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Receptors for Purines and Pyrimidines

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I. Introduction

A. Overview

Extracellular purines (adenosine, ADP, and ATP) and pyrimidines (UDP and UTP) are important signaling molecules that mediate diverse biological effects via cell-surface receptors termed purine receptors. In this review particular emphasis is placed on the discrepancy between the pharmacological properties of native and recombinant receptors for these agents.

There are two main families of purine receptors, adenosine or P1 receptors, and P2 receptors, recognizing primarily ATP, ADP, UTP, and UDP. Adenosine/P1 receptors have been further subdivided, according to convergent molecular, biochemical, and pharmacological evidence into four subtypes, A_1, A_{2A}, A_{2B}, and A_3, all of which couple to G proteins. Based on differences in molecular structure and signal transduction mechanisms, P2 receptors divide naturally into two families of ligand-gated ion channels and G protein-coupled receptors termed P2X and P2Y receptors, respectively; to date

[b] Abbreviations: ACh, acetylcholine; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; $ANAPP_3$, arylazidoaminopropionyl ATP; Ap_3A, P^1,P^3-diadenosine triphosphate; Ap_4A, P^1,P^4-diadenosine tetraphosphate; Ap_5A, P^1,P^5-diadenosine pentaphosphate; Ap_6A, P^1,P^6-diadenosine hexaphosphate; APEC, 2-[(2-aminoethylamino)carbonylethylphenylethylamino]-5′-N-ethylcarboxamido adenosine; APNEA, N-[2-(4-aminophenyl) ethyl] adenosine; ATP, adenosine 5′-triphosphate; A3P5P, adenosine-3′-phosphate-5′-phosphosulfate; ATPγS, adenosine 5′-O-(3-thiotriphosphate); BzATP, 3′-O-(4-benzoyl)benzoyl ATP; cAMP, adenosine 3′,5′-cyclic monophosphate; CGRP, calcitonin gene-related peptide; CGS 21680, 2-[*p*-(2-carbonyl-ethyl)-phenylethylamino]-5′-N-ethylcarboxamidoadenosine; CHO, chinese hamster ovary; CNS, central nervous system; CPA, N^6-cyclopentyladenosine; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonate; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; EDRF, endothelium-derived relaxing factor; EDHF, endothelium-derived hyperpolarizing factor; GRK, G protein-coupled receptor specific kinase; IB-MECA, N^6-(3-iodobenzyl)-5′-(N-methylcarbamoyl)adenosine; IP_3, inositol 1,4,5-trisphosphate; KFM 19, (±)-8-(3-oxocyclopentyl)-1,3-dipropylxanthine; MAPK, mitogen-activated protein kinase; α,β-meATP, α,β-methylene ATP; β,γ-meATP, β,γ-methylene ATP; 2MeSATP, 2-methylthio ATP; mRNA, messenger RNA; NECA, N-ethylcarboxamidoadenosine; NF023, symmetrical 3′-urea of 8-(benzamido)naphthalene-1,3,5-trisulfonic acid; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PNS, peripheral nervous system; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; R-PIA, (*R*)N^6-phenylisopropyl adenosine; RNA, ribonucleic acid; SCG, superior cervical ganglion; suramin, 8-(3-benzamido-4-methylbenzamido)-naphthalene-1,3,5-trisulfonic acid; 8-SPT, 8-(p-sulfophenyl)theophylline; TM, transmembrane; UDP, uridine 5′-diphosphate; UTP, uridine 5′-triphosphate; XAC, xanthine amine congener.

seven mammalian P2X receptors ($P2X_{1-7}$) and five mammalian P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$) have been cloned, characterized, and accepted as valid members of the P2 receptor family. As correlates between cloned and endogenous receptors are established, the structural subdivision will replace an earlier system of subclassification identifying endogenous P_{2X}, P_{2Y}, P_{2U}, P_{2T}, and P_{2Z} receptors principally according to their pharmacological profiles. A prominent issue addressed in this review is the apparent mismatch of pharmacological data in biological tissue relating to the P2 receptor subtypes classified on the basis of molecular structure. While it is logically satisfying to base receptor subclassification on amino acid sequencing where differences of 30 to 40% are generally regarded as justification for subtyping, it would seem that differences in sequence of less than 5% (even single point mutations) can result in substantial differences in pharmacological profile. Thus, receptor heterogeneity among species, together with receptor coexpression and the possible expression of new receptor subtypes that have not yet been cloned, complicates interpretation of pharmacological responses in some tissues. Thus, it will become apparent in the present review that, at least with the use of currently available, largely unselective agonists and antagonists, some response profiles do not fit those expected for the known P2 receptor subtypes.

B. Historical Perspective

Extracellular purines and pyrimidines have important and diverse effects on many biological processes including smooth muscle contraction, neurotransmission, exocrine and endocrine secretion, the immune response, inflammation, platelet aggregation, pain, and modulation of cardiac function. The concept of purines as extracellular signaling molecules was instigated by Drury and Szent-Györgyi in 1929, in a comprehensive report showing that adenosine and adenosine 5′-monophosphate (AMP), extracted from heart muscle, have pronounced biological effects, including heart block, arterial dilatation, lowering of blood pressure, and inhibition of intestinal contraction. Gillespie, in 1934, drew attention to the structure-activity relationships of adenine compounds, showing that deamination greatly reduces pharmacological activity, and that removal of the phosphates from the molecule influences not only potency, but also the type of response. Removal of phosphates was shown to increase the ability of adenine compounds to cause vasodilatation and hypotension, and ATP caused an increase in rabbit and cat blood pressure that was rarely or never observed with AMP or adenosine. Furthermore, ATP was shown to be more potent than AMP and adenosine in causing contraction of guinea-pig ileum and uterus (Gillespie, 1934). This was the first indication of different actions of adenosine and ATP and, by implication, the first indication of the existence of different purine receptors.

Early investigations into the effects of adenosine and ATP were made on a variety of tissues, but particularly the heart and vasculature (Gaddum and Holtz, 1933; Emmelin and Feldberg, 1948; Folkow, 1949; Green and Stoner, 1950). Initial studies on the effects of extracellular UTP also focused on its cardiovascular effects (Hashimoto *et al.*, 1964; Boyd and Forrester, 1968; Urquilla, 1978; Sakai *et al.*, 1979). Other early lines of purine research concerned the effects of purines on platelet aggregation (Born, 1962) and on mast cells (Cockcroft and Gomperts, 1980). Diverse responses to extracellular purines and pyrimidines have now been documented in a wide range of biological systems, from single cells to whole organisms, and include smooth muscle contraction, neurotransmission in the peripheral and central nervous system, exocrine and endocrine secretion, the immune response, inflammation, platelet aggregation, pain, and modulation of cardiac function (Burnstock and Kennedy, 1986; Gordon, 1986; Seifert and Schultz, 1989; Burnstock, 1990; Olsson and Pearson, 1990; Ralevic and Burnstock, 1991a; Jacobson *et al.*, 1992b; Dubyak and el-Moatassim, 1993; Dalziel and Westfall, 1994; Fredholm, 1995; Burnstock and Wood, 1996; Ongini and Fredholm, 1996; Sebastião and Ribeiro, 1996).

Insight into the physiological roles of extracellular purines and pyrimidines comes from studies of their biological sources and the stimuli for their release. In this respect, an important line of research stemmed from studies showing that adenosine is released from the heart during hypoxia to play an important role in reactive hyperemia (Berne, 1963; Gerlach *et al.*, 1963). The general hypothesis of coupling of purine release to metabolic demands via local regulation of blood flow has been applied to other tissues and includes the release of adenine nucleotides, particularly ATP, from skeletal muscle during contraction (Boyd and Forrester, 1968; Forrester and Lind, 1969).

Reports of ATP release from sensory nerves in the rabbit ear (Holton and Holton, 1953; Holton, 1959) were the first in a major line of research concerned with purines as neurotransmitters. ATP was detected in the venous perfusate following antidromic stimulation of the rabbit auricular nerve to elicit vasodilatation of the ear capillary bed, and both antidromic vasodilatation and vasodilatation to arterial infusion of ATP (but not that to other agents) were blocked by cholinesterase inhibitors (Holton and Holton, 1953; Holton, 1959). It is now known that ATP is an important neurotransmitter or cotransmitter and adenosine an important neuromodulator in both the peripheral and central nervous systems (see Stone, 1991; Burnstock, 1990; Edwards and Gibb, 1993; Fredholm, 1995). There are also hints that adenine dinucleotides may play neurotransmitter or neuromodulator roles in the central nervous system (Pintor and Miras-Portugal, 1995b).

Adrenal chromaffin granules (Cena and Rojas, 1990), platelets (Born and Kratzer, 1984; Gordon, 1986), mast cells (Osipchuk and Cahalan, 1992), erythrocytes (Forrester, 1990; Ellsworth *et al.*, 1995), basophilic leukocytes (Osipchuk and Cahalan, 1992), cardiac myocytes (Forrester, 1990), fibroblasts (Grierson and Meldolesi, 1995b), and endothelial (Ralevic *et al.*, 1991a, 1991c, 1995b; Bodin *et al.*, 1992) and epithelial cells (Enomoto *et al.*, 1994; Ferguson *et al.*, 1997) are important sources of purines that can be released under physiological and pathophysiological conditions, which may act on the purine receptors associated with these or neighboring cells. Adenine dinucleotides are released from secretory ganules of thrombocytes, chromaffin cells and neurons, and may represent a novel class of signaling molecules (Hoyle, 1990; Ogilvie, 1992; Ogilvie *et al.*, 1996). Not enough is known about the sources and release of pyrimidines, which limits our understanding of the role played by the widely distributed receptors that are activated by pyrimidines. However, steps toward resolving this are being made with the demonstration that UTP is released by physiologically relevant stimuli from cultured endothelial, epithelial, and astrocytoma cells (Enomoto *et al.*, 1994; Saiag *et al.*, 1995; Lazarowski *et al.*, 1997a).

Purines and pyrimidines mediate their effects by interactions with distinct cell-surface receptors. Early pharmacological evidence for the existence of adenosine receptors has been provided by specific antagonism by methylxanthines of adenosine-mediated accumulation of adenosine 3′,5′-cyclic monophosphate (cAMP) in rat brain slices (Sattin and Rall, 1970). "Purinergic" receptors were first formally recognized by Burnstock in 1978, when he proposed that these can be divided into two classes termed "P_1-purinoceptors", at which adenosine is the principal natural ligand, and "P_2-purinoceptors", recognizing ATP and ADP (Burnstock, 1978). This division was based on several criteria, namely the relative potencies of ATP, ADP, AMP, and adenosine, selective antagonism of the effects of adenosine by methylxanthines, activation of adenylate cyclase by adenosine, and stimulation of prostaglandin synthesis by ATP and ADP. This major division remains a fundamental part of purine receptor classification, although adenosine/P1 and P2 receptors are now characterized primarily according to their distinct molecular structures, supported by evidence of distinct effector systems, pharmacological profiles, and tissue distributions. In addition, receptors for pyrimidines are now included within the P2 receptor family (table 1) (Fredholm *et al.*, 1994, 1996). Note that it has been recommended that "P1 receptor" and "P2 receptor" replace the "P_1/P_2-purinoceptor" terminology (Fredholm *et al.*, 1996). The terms "adenosine receptor" and "P1 receptor" are synonymous.

Adenosine/P1 receptors are further divided into four subtypes, A_1, A_{2A}, A_{2B}, and A_3, on the basis of their distinct molecular structures and show distinct tissue distributions and pharmacological profiles. All couple to G proteins.

P2 receptors were shown to be ligand-gated cation channels (Benham and Tsien, 1987) or involved G protein activation (Dubyak, 1991), which formed the basis of their subdivision into two main groups termed P2X receptors (ligand-gated cation channels) and P2Y receptors (G protein-coupled receptors) (Abbracchio and Burnstock, 1994; Fredholm *et al.*, 1994). Subtypes are defined according to the molecular structure of cloned mammalian P2 receptors, discriminated by different numerical subscripts ($P2X_n$ or $P2Y_n$) (Burnstock and King, 1996; Fredholm *et al.*, 1996). This forms the basis of a system of nomenclature that will replace the earlier subtype nomenclature (including P_{2X}, P_{2Y}, P_{2U}, P_{2T}, and P_{2Z} receptors) as correlations between cloned and endogenous receptors are established. P3, P4, and $P2Y_{Ap4A}$ (or P_{2D}) receptors have been proposed, but evidence for their existence is based solely on the distinct pharmacological profiles exhibited by some biological tissues. As this is no longer tenable for the identification and subclassification of receptors, it remains to be determined, preferably by molecular studies, how these correlate with cloned P2 receptors, and therefore exactly how they will fit within a unifying system of purine and pyrimidine receptor nomenclature.

TABLE 1
Families of receptors for purines and pyrimidines

	Adenosine/P1 receptors	P2 receptors	
Natural ligands	Adenosine	ATP ADP UTP UDP Adenine dinucleotides	
Subgroup	—	P2X	P2Y
Type	G protein-coupled	Ion channel Nonselective pore[a]	G protein-coupled
Subtypes	A_1, A_{2A}, A_{2B}, A_3	$P2X_{1-7}$, $P2X_n$	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{ADP}$ (or P_{2T}) Uridine nucleotide-specific[b]

AMP does not activate P2 receptors, but may be an agonist at adenosine/P1 receptors.
P_{2Xn}, heteropolymeric receptors such as $P2X_2P2X_3$ and possibly others with subunit combinations currently unknown.
[a] P_{2X7} (or P_{2Z}) receptor only.
[b] Endogenous uridine nucleotide-specific receptors, which may have counterparts in $P2Y_4$ and $P2Y_6$ receptors.

The main aim of this review is to present the characteristics of receptors for purines and pyrimidines within a framework whereby comparisons can be made between cloned and endogenous receptors. For the P2 receptor family this is in order to promote the conversion from a system of nomenclature that is rapidly losing its value, to a unifying system of classification based on structurally distinct cloned receptors. However, pharmacological characterization of endogenous P2 receptors is often equivocal, largely because of the current lack of selective agonists and antagonists and because of complications introduced by the common and widespread coexpression of different types of P2 receptors. Thus, it will become apparent in the present review that in assigning names to endogenous P2 receptors we have needed to strike a balance between caution (against overinterpretation) and anticipation of the direction in which this field is heading. Signal transduction mechanisms, pharmacological response profiles, receptor desensitization, tissue distribution, and biological effects of receptors for purines and pyrimidines are considered. Because ATP and ADP are rapidly degraded to adenosine, and because most cells and tissues coexpress P1 and P2 receptors, we also consider the interactions that may occur between receptors belonging to these two families. Although modulation of ecto-nucleotidase expression and activity is an important means by which to regulate purine receptor function, this aspect of purinergic signaling is not dealt with in detail in this article; the reader is referred to other reviews on the subject (Ziganshin *et al.*, 1994a; Zimmerman, 1996).

II. Adenosine/P1 Receptors

A. Introduction

The adenosine/P1 receptor family comprises A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors, identified by convergent data from molecular, biochemical, and pharmacological studies (table 2). Receptors from each of these four distinct subtypes have been cloned from a variety of species and characterized following functional expression in mammalian cells or *Xenopus* oocytes (table 3). A_1 and A_2 receptors were described by Van Calker *et al.* in 1979, in studies showing that activation of these receptors by adenosine and its derivatives inhibited, via A_1, or stimulated, via A_2, adenylate cyclase activity in cultured mouse brain cells (Van Calker *et al.*, 1979). The effects of adenosine could be antagonized by methylxanthines and the order of potency of adenosine analogs was different for the two receptors (Van Calker *et al.*, 1979). Londos *et al.* (1980) independently drew similar conclusions using membrane preparations from rat adipocytes, hepatocytes, and Leydig tumor cells; the adenosine receptors coupled to inhibition of adenylate cyclase (those in adipocytes) they named R_i (corresponding to the A_1 subtype) and the adenosine receptors coupled to stimulation of adenylate cyclase (those in hepatocytes and Leydig cells) they termed R_a (synonymous with the A_2 subtype). This alternative system of nomenclature was based on "R" to designate the "ribose" moiety of the nucleoside and "i" and "a" to indicate inhibition and activation of adenylate cyclase respectively (Londos *et al.*, 1980). The A_1/A_2 nomenclature is now used, which is fortunate because A_1 receptors act through a variety of transduction mechanisms in addition to adenylate cyclase. A_{1a} and A_{1b} receptors have been proposed (Gustafsson *et al.*, 1990), but this subdivision of the A_1 receptor remains equivocal.

A_2 receptors are further subdivided into types A_{2A} and A_{2B}. The suggestion that A_2 receptors could be divided into two classes was originally based on the recognition that adenosine-mediated stimulation of adenylate cyclase in rat brain was effected via distinct high affinity

TABLE 2
Classification of adenosine/P1 receptors

	A_1	A_{2A}	A_{2B}	A_3
G protein-coupling	$G_{i/o}$	G_s	G_s G_q	G_i G_q
Effects	↓ cAMP ↑ IP_3 ↑ K^+ ↓ Ca^{2+}	↑ cAMP	↑ cAMP ↑ IP_3	↓ cAMP ↑ IP_3
Selective agonists	CPA, CCPA, CHA, *R*-PIA	CGS21680, HE-NECA, APEC, CV 1808, DPMA, WRC-0470	—	IB-MECA, 2Cl-IB-MECA
Selective antagonists	DPCPX, XAC, KW-3902, ENX, KFM 19, N 0861, FK 453, WRC 0571	KF17837, ZM241385, CSC, SCH 58261	—	I-ABOPX[a], L-268605, L-249313, MRS 1067, MRS 1097

Abbreviations: APEC, 2-[(2-aminoethylamino)carbonylethylphenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; CGS21680, 2-[*p*-(2-carbonyl-ethyl)-phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; CCPA, 2-chloro-CPA; CHA, N^6-cyclopentyladenosine; 2Cl-IB-MECA, 2-chloro-N^6-(3-iodobenzyl)-5′-(N-methylcarbamoyl)adenosine; CPA, N^6-cyclopentyladenosine; CSC, 8-(3-chlorostyry)caffeine; CV 1808, 2-phenylaminoadenosine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; DPMA, N^6-[2(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]-adenosine; ENX, 1,3-dipropyl-8-[2-(5,6-epoxy)norbornyl]xanthine; FK 453, (+)-(*R*)-[(*E*)-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)acryloyl]-2-piperidine ethanol; HE-NECA, 2-hexyl-5′-N-ethylcarboxamidoadenosine; I-ABOPX, 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propylxanthine; IB-MECA, N^6-(3-iodobenzyl)-5′-(N-methylcarbamoyl)adenosine; KF17837, 1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine; KFM 19, [(±)-8-(3-oxocyclopentyl)-1,3-dipropylxanthine]; KW-3902, 8-noradamant-3-yl-1,3-dipropylxanthine; L-249313, 6-carboxymethyl-5,9-dihydro-9-methyl-2-phenyl-[1,2,4]-triazolo[5,1-*a*][2,7]naphthyridine; L-268605, 3-(4-methoxyphenyl)-5-amino-7-oxo-thiazolo[3,2]pyrimidine; MRS 1067, 3,6-dichloro-2′-isopropyloxy-4′-methylflavone; MRS 1097, 3,5-diethyl 2-methyl-6-phenyl-4-(*trans*-2-phenylvinyl)-1,4(*R,S*)-dihydropyridine-3,5-dicarboxylate; N 0861, 1,3-dipropyl-8-[2,(5,6-epoxy)norbornyl]xanthine; *R*-PIA, (*R*)N^6-phenylisopropyladenosine; SCH 58261, 5-amino-7-(2-phenylethyl)-2 (2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; WRC 0470, 2-cyclohexylmethylidenehydrazinoadenosine; WRC 0571, 8-(N-methylisopropyl)amino-N^6-(5′-endohydroxy-endonorbornyl)-9-methyladenine; XAC, xanthine amine congener; ZM 241385, 4-(2-[7-amino-2-(2-furyl)]1,2,4-triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol.

[a] High affinity (nM) at sheep and human, but not rat A_3 adenosine receptors.

TABLE 3
Cloned adenosine/P1 receptors

	Number of amino acids	cDNA library source	References
A_1	326	Human brain	Libert *et al.*, 1992; Townsend-Nicholson and Shine, 1992
	326	Canine thyroid	Libert *et al.*, 1989, 1991
	326	Bovine brain	Tucker *et al.*, 1992; Olah *et al.*, 1992
	328	Rabbit kidney	Bhattacharya *et al.*, 1993
	326/327	Rat brain	Reppert *et al.*, 1991; Mahan *et al.*, 1991
	326	Mouse brain	Marquardt *et al.*, 1994
	326	Guinea-pig brain	Meng *et al.*, 1994a
A_{2A}	409	Human hippocampus	Furlong *et al.*, 1992
	411	Canine thyroid	Libert *et al.*, 1989; Maenhaut *et al.*, 1990
	410	Rat brain	Chern *et al.*, 1992; Fink *et al.*, 1992
	409	Guinea-pig brain	Meng *et al.*, 1994b
	410	Mouse bone marrow-derived mast cells	Marquardt *et al.*, 1994
A_{2B}	328	Human hippocampus	Pierce *et al.*, 1992
	332	Rat brain	Stehle *et al.*, 1992; Rivkees and Reppert, 1992
	332	Mouse bone marrow-derived mast cells	Marquardt *et al.*, 1994
A_3	318	Human striatum	Salvatore *et al.*, 1993
	318	Human heart	Sajjadi *et al.*, 1993
	317	Sheep pars tuberalis	Linden *et al.*, 1993
	320	Rabbit lung	Hill *et al.*, 1997
	320	Rat brain	Zhou *et al.*, 1992
	320	Rat testis	Meyerhof *et al.*, 1991; Zhou *et al.*, 1992

binding sites (localized in striatal membranes) and low affinity binding sites (present throughout the brain) (Daly *et al.*, 1983). This subdivision was supported in a study which compared the high affinity striatal A_2 binding site with a low-affinity A_2 binding site characterized in a human fibroblast cell line; the two sites were termed A_{2A} and A_{2B}, respectively (Bruns *et al.*, 1986). Definitive evidence for the existence of these two subtypes comes from the cloning and sequencing of distinct A_{2A} and A_{2B} receptors which show distinct pharmacological profiles in transfected cells similar to those of the endogenous receptors.

Consistent with the fact that these are distinct receptors, there is a considerable lack of amino acid sequence homology between cloned A_1, A_{2A}, A_{2B}, and A_3 receptors. For example, the homology between rat A_1 and A_{2B} receptors is only 45% (Stehle *et al.*, 1992), and the human A_3 receptor only shows approximately 50%, 43%, and 40% homology with human A_1, A_{2A}, and A_{2B} receptors, respectively (Linden, 1994). The homology between A_{2A} and A_{2B} receptors is also slight, being approximately 46% when these subtypes are cloned from rat and 61% when cloned from human (Stehle *et al.*, 1992; Pierce *et al.*, 1992).

An adenosine binding site with high affinity for 2-phenylaminoadenosine (CV 1808) (A_{2A}-selective agonist) in rat striatal membranes has been suggested to be a novel "A_4" adenosine receptor (Cornfield *et al.*, 1992). The very low affinity of 2-[p-(2-carbonyl-ethyl)-phenylethylamino]-5'-N-ethylcarboxamidoadenosine (CGS 21680) and N-ethylcarboxamidoadenosine (NECA) at this site were taken to indicate that this is not an A_2 receptor. However, the binding studies were carried out at 4°C (Cornfield *et al.*, 1992), and the existence of a distinct A_4 receptor has been challenged by the demonstration that when similar binding studies are carried out at 21°C, the potency order of compounds at the striatal membrane site is characteristic of the A_{2A} adenosine receptor (Luthin and Linden, 1995). Furthermore, in COS cells transfected with adenosine A_{2A} receptors, binding studies carried out at 4°C produce an "A_4" binding profile (Luthin and Linden, 1995). This justifies the more rigorous criteria now demanded for classification of receptors, whose identity must be proved by molecular as well as by biochemical or pharmacological means.

There is a vast and rapidly growing literature on adenosine/P1 receptors; it has not been possible to do justice to this in the present review. Out of necessity, therefore, we focus on the more recent literature.

B. Structure

All adenosine receptors couple to G proteins. In common with other G protein-coupled receptors, they have seven putative transmembrane (TM) domains of hydrophobic amino acids, each believed to constitute an α-helix of approximately 21 to 28 amino acids. The N-terminal of the protein lies on the extracellular side and the C-terminal on the cytoplasmic side of the membrane. A pocket for the ligand binding site is formed by the three-dimensional arrangement of the α-helical TM domains, and the agonist is believed to bind within the upper half of this pore. The transmembrane domains are connected by three extracellular and three cytoplasmic hydrophilic loops of unequal size; typically the extracellular loop between TM4 and TM5 and the cytoplasmic loop between TM5 and TM6 is extended. These features are illustrated in a schematic of the A_1 receptor in figure 1.

N-linked glycosylation often occurs on the second extracellular loop; the roles of the carbohydrate moieties of the glycosylated receptor are not clear, although suggested functions include stabilization of protein conformation, protection of proteins from proteases, and mod-

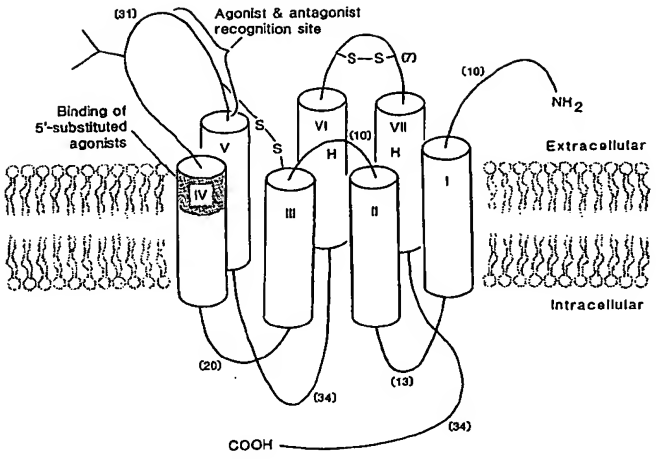


FIG. 1. Schematic of the A_1 adenosine receptor. In common with other G protein-coupled receptors, the A_1 receptor has seven putative transmembrane domains (I-VII) of hydrophobic amino acids, each believed to constitute an α-helix, which are connected by three extracellular and three intracellular hydrophilic loops. The number of amino acids comprising the extra- and intracellular loops and the extracellular N-terminal and intracellular C-terminal regions of the bovine A_1 receptor are indicated in parentheses (Olah *et al.*, 1992). The transmembrane regions comprise 23 to 25 amino acids in the bovine A_1 receptor (Olah *et al.*, 1992). The arrangement of the transmembrane regions forms a pocket for the ligand binding site. The location of histidine residues (H) in transmembrane regions VI (position 254) and VII (position 278) in the bovine A_1 receptor, which are believed to be important in ligand binding (Olah *et al.*, 1992), are indicated. Extracellular and transmembrane regions of the protein believed to be important in agonist and antagonist binding are indicated (Olah *et al.*, 1994b,c). S-S denotes the presence of hypothetical disulfide bridges (Jacobson *et al.*, 1993c). Glycosylation occurs on the second extracellular loop.

ulation of protein function. Current evidence suggests that glycosylation has no obvious influence on ligand binding (Piersen *et al.*, 1994). The intracellular segment of the receptor interacts with the appropriate G protein with subsequent activation of the intracellular signal transduction mechanism. The third intracellular loop of the adenosine A_{2A} receptor seems to be the main determinant of its G protein selectivity (Olah, 1997). Phosphorylation by protein kinases of amino acid residues on the cytoplasmic domains seems to be involved in desensitization of A_{2A} and A_3 receptors (Palmer and Stiles, 1997a, 1997b).

The transmembrane regions are generally highly conserved, with particularly long stretches of amino acid homology being found in TM2, TM3, and TM5. Most sequence differences have been observed in a hypervariable region located at the N-terminal half of the second extracellular loop (Tucker and Linden, 1993). It is the residues within the transmembrane regions that are crucial for ligand binding and specificity and, with the exception of the distal (carboxyl) region of the second extracellular loop, the extracellular loops, the C-terminal and the N-terminal do not seem to be involved in ligand recognition (Olah *et al.*, 1994b, 1995). A number of amino acid residues contribute, in different ways, to ligand specificity within the binding pocket. Site-directed mutagenesis of the bovine A_1 adenosine receptor suggests that conserved histidine residues in TM6 and TM7 are important in ligand binding. Histidine 278 in TM7 seems to be particularly important because mutation of this amino acid abolishes ligand binding (Olah *et al.*, 1992). Mutagenesis of the human A_1 adenosine receptor has shown that threonine 277 in TM7 is important in binding of the non-selective adenosine receptor agonist NECA, but has little effect on the affinity of binding of the A_1 selective agonist (R)-N^6-(2-phenyl-1-methylethyl)-adenosine (R-PIA), or of antagonists (Townsend-Nicholson and Schofield, 1994). Modification of Glu 16 in TM1 and Asp 55 in TM2 of the human A_1 receptor alters the affinity of binding for [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) and other agonists, but does not affect antagonist binding (Barbhaiya *et al.*, 1996). Site-directed mutagenesis of the human A_{2A} adenosine receptor has identified several residues in TM5–7 that are involved in ligand binding (Kim *et al.*, 1995). Glu 13 in TM1 of the human A_{2A} receptor seems to be critically involved in agonist, but not antagonist recognition (Ijzerman *et al.*, 1996).

A potential problem inherent in the methodology of site-directed mutagenesis is that changes in primary structure may cause changes in tertiary structure of the molecule. This has been addressed by studies with chimeras constructed from structurally similar, but pharmacologically different receptors. The ligand binding properties of A_1/A_3 chimeric receptors support the concept of a crucial role for histidine residues in TM6 and TM7 in ligand binding (Olah *et al.*, 1995). In addition, a critical role in ligand binding of the distal region of the second extracellular loop has been identified, although its specific interactions are not yet clear (Olah *et al.*, 1994b). Possible roles include direct interaction of an amino acid residue(s) within this region with the ligand, an influence on the conformation of the receptor and/or steric hindrance. Construction of chimeric human A_1 and rat A_{2A} adenosine receptors was used to show that TM1–4 are important in A_1 receptor agonist and antagonist binding and ligand specificity (Rivkees *et al.*, 1995a).

C. Agonists

Analogs with greater stability than adenosine are produced by modification of the N^6 and C2 positions of the adenine ring and the 5′-position of the ribose moiety of adenosine, and have been used extensively in the characterization of adenosine/P1 receptors. NECA (Williams, 1989), N-[2-(4-aminophenyl)ethyl] adenosine (APNEA) (Fozard and Carruthers, 1993), and N^6-(3-[^{125}I]iodo-4-aminobenzyl)-5′-N-methylcarboxamidoadenosine (^{125}I-AB-MECA) (Olah *et al.*, 1994a) do not discriminate between adenosine receptor subtypes. Agonists with subtype selectivity are detailed in the sections on individual adenosine receptor subtypes and the chemical structure of some of these are illustrated in figure 2.

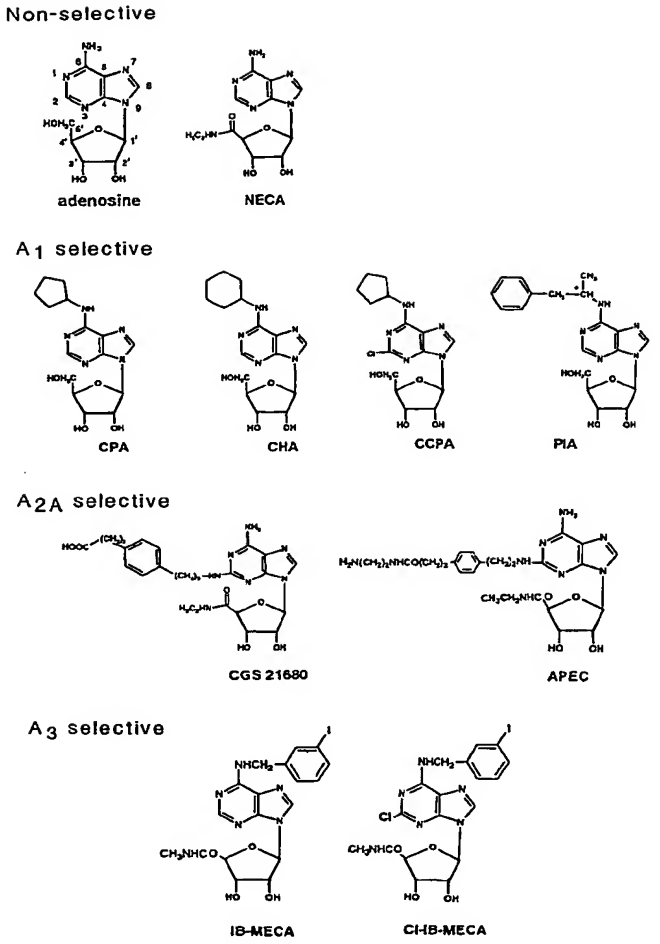


FIG. 2. The chemical structure of some agonists at adenosine/P1 receptors.

ATP and metabolically stable ATP derivatives, i.e., adenosine 5′-O-(3-thiotriphosphate)(ATPγS) and β,γ-methylene ATP (β,γ-meATP), can act directly as agonists at adenosine/P1 receptors in some tissues where responses are blocked by methylxanthines, but are not affected by adenosine deaminase or by blockade of 5′-nucleotidase. β,γ-MeATP is approximately equipotent with adenosine at mediating contraction of smooth muscle adenosine/P1 receptors of rat colon (Bailey and Hourani, 1990), and relaxation via adenosine/P1 receptors of rat duodenum (Hourani *et al.*, 1991), and guinea-pig trachealis muscle (Piper and Hollingsworth, 1996). ATP, ATPγS, and β,γ-meATP inhibit [^{3}H]-NA release in a variety of tissues via receptors that are blocked by the A_1 selective antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) as well as by the P2 receptor antagonist cibacron blue (Von Kügelgen *et al.*, 1992, 1995b, 1996). ATP (Collis and Pettinger, 1982) and diadenosine polyphosphates (Hoyle *et al.*, 1996; Vahlensieck *et al.*, 1996) have been reported to stimulate directly adenosine/P1 receptors in guinea-pig atria, eliciting negative inotropic and chronotropic effects without prior conversion to adenosine. These effects are not consistent with the pharmacological profile of any of the established subtypes of adenosine/P1 receptor, and in some respects are similar to the profile described for the P3 receptor.

D. Antagonists

Xanthines and xanthine derivatives, including the natural derivatives theophylline and caffeine, are non-selective adenosine/P1 receptor antagonists. They are not universal adenosine/P1 receptor antagonists; xanthine-resistant relaxations to adenosine and its analogs were observed in guinea-pig aorta (Collis and Brown, 1983; Martin, 1992), rat aorta (Prentice and Hourani, 1996), guinea-pig trachea (Brackett and Daly, 1991), porcine coronary artery (Abebe *et al.*, 1994), and guinea-pig taenia cecum (Prentice *et al.*, 1995). Some A_3 receptors, namely those of rat, rabbit, and gerbil, are characteristically insensitive to methylxanthines, thus it is possible that the xanthine-resistant responses to adenosine described in some tissues occur following actions of adenosine at mast cell A_3 receptors and the subsequent release of vasoactive mediators. This hypothesis would predict that guinea-pig and pig A_3 receptors are also xanthine-insensitive, because xanthine-resistant responses to adenosine have been reported in these species. It would be interesting to see if these responses can be blocked by inhibitors of mast cell degranulation.

8-Phenyltheophylline and the more water soluble 8-(p-sulfophenyl)theophylline (8-SPT) (Daly *et al.*, 1985) are more potent than theophylline at adenosine/P1 receptors, but are not subtype-selective. 8-SPT and its derivative 1,3-dipropyl-8-sulfophenylxanthine (DPSPX) do not cross the blood-brain barrier, being purely peripherally acting adenosine/P1 receptor antagonists (Daly *et al.*, 1985) and thus can be used to discriminate between central and peripheral adenosine receptors. A number of xanthines and non-xanthines identified as adenosine receptor antagonists with reasonable subtype selectivity are described below (see Sections III.F., IV.F., and VI.F.) and their chemical structures illustrated in figure 3.

III. A_1 Receptor

Subdivision of A_1 receptors into high affinity A_{1a} receptors and low affinity A_{1b} receptors has been proposed (Gustafsson *et al.*, 1990). This was based on the description of high-affinity binding sites for adenosine agonists and antagonists in rat and guinea-pig brain (A_{1a}) and low-affinity binding sites in rat vas deferens and guinea-pig ileum (A_{1b}) (Gustafsson *et al.*, 1990). However, there are no cloned equivalents for these putative subtypes and their existence remains equivocal. It is possible that these reflect high and low affinity states of the same A_1 receptor.

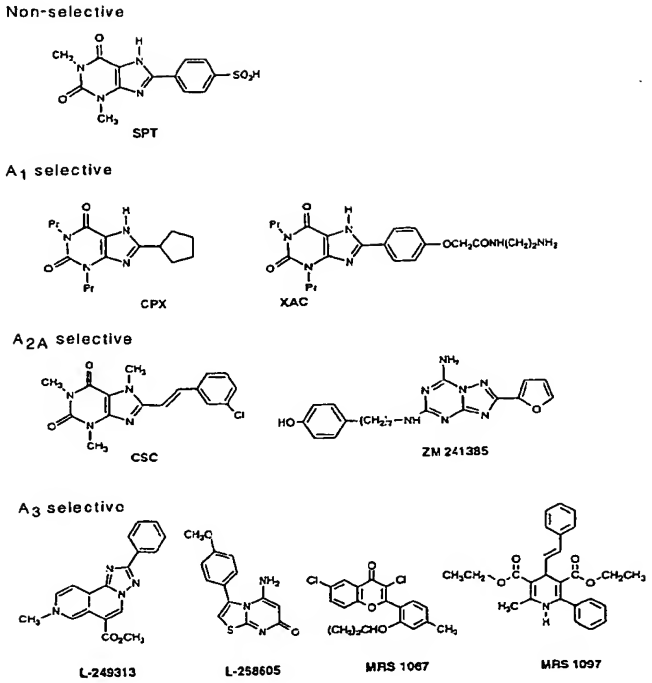


FIG. 3. The chemical structure of some antagonists at adenosine/P1 receptors.

A. Cloned A_1 Receptors

A_1 receptors have been cloned from several species (table 3). The human adenosine A_1 receptor subtype gene (ADORA1) has been localized to chromosome 1q32.1 (Townsend-Nicholson *et al.*, 1995a). The variability in the primary sequence of the A_1 receptor between species is less than 10% for A_1 receptors from dog, rat, and cow, and less than 5% between bovine and human A_1 receptors, but this seems to be sufficient to cause considerable interspecies differences in ligand binding (Tucker and Linden, 1993) and subtle differences in the mechanisms underlying receptor desensitization (Ramkumar *et al.*, 1991; Nie *et al.*, 1997; Palmer and Stiles, 1997b). Species homologs of A_1 receptors have been suggested to differ in their ability to discriminate among the related G_o/G_i protein alpha subunits (Jockers *et al.*, 1994).

B. Signal Transduction Mechanisms

The A_1 receptor mediates a broad range of signaling responses, which may be caused by its coupling to different G proteins within the $G_{i/o}$ family (Freissmuth *et al.*, 1991; Munshi *et al.*, 1991). The G proteins G_i and G_o are substrates for pertussis toxin that ADP-ribosylates the α-subunit of $G_{i/o/t}$ family members, uncoupling them from receptors. Accordingly, effects mediated by A_1 receptors are generally blocked by pertussis toxin. However, presynaptic A_1 receptors seem to be at least partly resistant to pertussis toxin (Fredholm *et al.*, 1989; Hasuo *et al.*, 1992); the reason for this could be the very tight coupling of the presynaptic A_1 receptors to potentially pertussis toxin-sensitive G proteins, rather than coupling to pertussis toxin-insensitive G proteins (Van der Ploeg *et al.*, 1992). A partially-purified protein with selectivity for G protein α subunits has been shown to stabilize the rat brain A_1 receptor-G protein complex, thereby promoting tight coupling of the A_1 receptor with its G protein (Nanoff *et al.*, 1997). Interestingly, this is a feature of the rat brain but not the human brain A_1 receptor; the latter is not under the control of a coupling cofactor, but operates according to the classic ternary complex model of receptor-G protein coupling (Nanoff *et al.*, 1997).

The most widely recognized signaling pathway of A_1 receptors is inhibition of adenylate cyclase causing a decrease in the second-messenger cAMP (Van Calker *et al.*, 1978; Londos *et al.*, 1980). This in turn modulates the activity of cAMP-dependent protein kinase, which phosphorylates diverse protein targets. A_1 coupling to adenylate cyclase has been described in a number of tissues including brain, adipose tissue, and testes. In addition to direct modulation of signaling pathways downstream to cAMP, inhibition of adenylate cyclase via A_1 receptors blocks the effects of other agents which act by stimulating adenylate cyclase activity in cells.

Another signaling mechanism of A_1 receptors is activation of phospholipase C (PLC) leading to membrane phosphoinositide metabolism and increased production of inositol 1,4,5-triphosphate (IP_3) [and diacylglycerol (DAG)] and Ca^{2+} mobilization. This has been described in chinese hamster ovary (CHO)-K1 cells expressing the cloned human A_1 receptor (Iredale *et al.*, 1994; Megson *et al.*, 1995) as well as at endogenous A_1 receptors in a number of tissues including DDT_1 MF-2 smooth muscle cells (Gerwins and Fredholm, 1992a,b; White *et al.*, 1992), heart (Scholz *et al.*, 1993), myometrium (Schiemann *et al.*, 1991a,b), rabbit cortical collecting tubule cells (Arend *et al.*, 1989), renal cells (Weinberg *et al.*, 1989), tracheal epithelial cells (Galietta *et al.*, 1992), cultured mesangial cells (Olivera *et al.*, 1992), and primary astrocytes (Peakman and Hill, 1995). IP_3 stimulates the release of Ca^{2+} from intracellular stores via interactions with specific receptors located on the sarcoplasmic reticulum. Elevation of cytosolic Ca^{2+} by IP_3 can stimulate a variety of signaling pathways, including a family of phosphatidyl serine-dependent serine/threonine-directed kinases collectively called protein kinase C (PKC) (of which there are at least 11 different isoforms), phospholipase A_2 (PLA_2), Ca^{2+}-dependent K^+ channels, and nitric oxide synthase (NOS). Depletion of Ca^{2+} from IP_3-sensitive pools may promote Ca^{2+} influx from extracellular sources.

Activation of phospholipase D (PLD) via A_1 adenosine receptors in DDT_1 MF-2 smooth muscle cells has been described (Gerwins and Fredholm, 1995a, 1995b), although as in the majority of cell systems this may be

downstream of phosphoinositide hydrolysis and may require the intermediate activation of PKC or Ca^{2+}.

Stimulation of A_1 receptors can activate several types of K^+ channel, described principally in cardiac muscle and neurons. In supraventricular tissues (sino-atrial and atrioventricular node, and atrium), the A_1 receptor couples directly via pertussis toxin-sensitive G proteins to K^+ channels (the same K^+ channels are activated by both adenosine and acetylcholine), and activation causes bradycardia (Belardinelli *et al.*, 1995a; Bünemann and Pott, 1995; Ito *et al.*, 1995). A_1 adenosine receptors also couple to ATP-sensitive K^+ channels (K_{ATP} channel); the activity is additionally regulated by metabolic demand (they close when intracellular ATP levels are high). Coupling seems to occur through the G protein in a membrane-delimited manner (Kirsch *et al.*, 1990; Dart and Standen, 1993), although coupling via cytosolic factors is possible given the strong evidence that A_1 receptors, K_{ATP} channels, and PKC all have a role in ischemic preconditioning. A_1 receptor coupling to K_{ATP} channels has been described in rat and guinea-pig ventricular myocytes (Kirsch *et al.*, 1990; Ito *et al.*, 1994), porcine coronary arteries (Merkel *et al.*, 1992; Dart and Standen, 1993), rabbit heart (Nakhostine and Lamontagne, 1993), and rat cerebral cells (Heurteaux *et al.*, 1995). Activation of K_{ATP} channels mediates a reduction in action potential duration, vasodilatation and an increase in blood flow, which is consistent with their having a pivotal role in the coupling of vascular tone to metabolic demand determined both by intracellular purines (ATP/ADP levels) and by the extracellular actions of adenosine (released, for instance, during hypoxia or ischemia).

Neurons express multiple K^+ channels that A_1 receptors may couple to regulate membrane potential and determine action potential frequency and duration. A_1 receptors reduce neuronal excitability and decrease firing rate by a hyperpolarizing effect mediated mainly by an increase in K^+ conductance (Trussell and Jackson, 1985; Greene and Haas, 1991; Pan *et al.*, 1995).

A_1 receptors also couple to inhibition of Ca^{2+} currents, which may account for inhibition of neurotransmitter release, although other or multiple mechanisms may be involved in this process (see Fredholm, 1995). Inhibition of Ca^{2+} currents by A_1 receptors has been described in dorsal root ganglion neurons (Dolphin *et al.*, 1986), rat hippocampal pyramidal neurons (Scholz and Miller, 1991), rat sympathetic neurons (N-type Ca^{2+} channels, plus an unidentified Ca^{2+} channel) (Zhu and Ikeda, 1993), rat brainstem (predominantly N-type, but also P-type Ca^{2+} channels) (Umemiya and Berger, 1994), hippocampal CA1 neurons (N-type, plus some unidentified Ca^{2+} channels) (Wu and Saggau, 1994), hippocampal CA3 neurons (N-type Ca^{2+} channel) (Mogul *et al.*, 1993), and mouse motoneurons (N-type Ca^{2+} channel) (Mynlieff and Beam, 1994). In atrial myocytes adenosine has an inhibitory effect on basal L-type Ca^{2+} current, although this is small and may be secondary to a reduction in basal cAMP (Belardinelli *et al.*, 1995a).

C. Desensitization

Several mechanisms, operational at different levels of the signal transduction cascade, contribute to differential desensitization of G protein-coupled receptors. Rapid desensitization (occuring within a few minutes of agonist exposure) seems to involve phosphorylation of specific residues on the receptor C-terminal or the cytoplasmic loops by G protein-coupled receptor-specific kinases (GRKs) and/or kinases regulated by levels of intracellular second-messengers such as cAMP-dependent protein kinase. The phosphorylated receptor may bind to members of a family of proteins called arrestins, which cause uncoupling of the receptor from its G proteins. Desensitization occuring over a longer time course also involves uncoupling of the receptor-G proteins complex, but phosphorylation does not seem to be a prerequisite. Sequestration of receptors into an intracellular compartment may occur, as described for the increase in A_1 receptors in light vesicle membrane fractions prepared from the hamster vas deferens smooth muscle cell line, DDT_1 MF-2 cells, after chronic exposure to R-PIA (Ramkumar *et al.*, 1991). Prolonged exposure to agonist may additionally lead to down-regulation of receptors and/or of the associated G proteins.

Desensitization of A_1 receptors by exposure to adenosine analogs has consistently been described both in vitro and in vivo, but this usually requires prolonged exposure to agonist (from 15 minutes to several hours or even days) (Parsons and Stiles, 1987; Ramkumar *et al.*, 1991; Abbracchio *et al.*, 1992; Green *et al.*, 1992; Lee *et al.*, 1993; Longabaugh *et al.*, 1989; Casati *et al.*, 1994). This is considerably longer than the time to desensitization of A_3 receptors which typically undergo significant desensitization within several minutes. Interestingly, while an agonist-stimulated increase in phosphorylation has been described for A_1 receptors in hamster DDT_1 MF-2 cells in association with receptor uncoupling from G proteins and desensitization, presumably by GRKs (Ramkumar *et al.*, 1991; Nie *et al.*, 1997), phosphorylation does not occur for the human A_1 receptor expressed in CHO cells at a time when receptor down-regulation is observed (Palmer and Stiles, 1997b). Down-regulation of A_1 receptors and/or of the associated G proteins after prolonged exposure to agonist has been reported in most of the cells and tissues in which this has been studied (Parsons and Stiles, 1987; Longabaugh *et al.*, 1989; Green *et al.*, 1992; Ramkumar *et al.*, 1991, 1993a; Abbracchio *et al.*, 1992).

Down-regulation of G proteins following A_1 receptor activation may lead to heterologous receptor desensitization. Chronic stimulation of A_1 receptors in adipocytes in vivo (Longabaugh *et al.*, 1989) and in isolated adipocytes (Green *et al.*, 1992) with (-)N^6-phenylisolpropyl adenosine (PIA) for up to 6 and 7 days, respectively,

causes down-regulation of A_1 receptors, non-uniform down-regulation of G_i proteins, and heterologous desensitization of other lipolytic hormone responses. In contrast, chronic (7 days) infusion of (R)N^6-phenylisopropyl adenosine (R-PIA) in guinea-pigs homologously desensitizes the atrioventricular nodal response to adenosine: there is down-regulation of A_1 adenosine receptors, a decrease in high affinity A_1 receptors, and a decrease in G_i and G_o proteins, but no change in responses mediated by muscarinic receptors (Dennis *et al.*, 1995).

D. Sensitization / Up-Regulation

Long-term treatment with adenosine/P_1 receptor antagonists generally leads to an increase in the effects of adenosine via a selective increase in the number of A_1 receptors, receptor sensitization and/or altered interaction between the receptor and the associated G proteins (Fredholm, 1982; Murray, 1982; Fredholm *et al.*, 1984; Green and Stiles, 1986; Ramkumar *et al.*, 1991; Fastbom and Fredholm, 1990; Zhang and Wells, 1990; Lupica *et al.*, 1991a, 1991b; Shi *et al.*, 1994). Long-term (12 day) caffeine treatment of rats increases the number of hippocampal A_1 (but not A_{2A}) receptors, without any changes in A_1 messenger ribonucleic acid (mRNA), suggesting that the adaptive changes are at the posttranslational level (Johansson *et al.*, 1993a). An increase in the density of cortical A_1 receptors has been described after chronic caffeine injestion in mice, but surprisingly, given that striatal adrenergic, cholinergic, GABA, and serotonin receptors and Ca^{2+} channels are also affected by this treatment, there is no change in the density of striatal A_{2A} receptors (Shi *et al.*, 1993).

E. Agonists

Certain N^6-substituted adenosine derivatives, such as N^6-cyclopentyladenosine (CPA), N^6-cyclohexyladenosine (CHA), and R-PIA, are selective agonists at A_1 receptors with K_i values in the range of 0.6 to 1.3 nM (see Jacobson *et al.*, 1992b) (table 2).

Substitutions at both the N^6- and C2-positions have produced 2-chloro-CPA (CCPA) which is A_1 selective, 1500-fold versus A_2 receptors in binding studies in rat brain, with a K_i of 0.6 nM (Lohse *et al.*, 1988; Thompson *et al.*, 1991; Jacobson *et al.*, 1992b). N-[1S, *trans*,2-hydroxycyclopentyl] adenosine (GR79236) has been reported to be an A_1 selective agonist, which is approximately equipotent with CPA in a variety of isolated tissues and cell types (Reeves *et al.*, 1993; Gurden *et al.*, 1993).

F. Antagonists

Most of the selective A_1 receptor antagonists described to date are xanthine-based derivatives. The introduction of hydrophobic (particularly phenyl or cycloalkyl) substituents into position 8 of the xanthine ring has yielded potent and A_1-selective antagonists, including 1,3-dipropyl-8-phenyl(2-amino-4-chloro)xanthine (PACPX), DPCPX, and xanthine amine congener (XAC) (Bruns *et al.*, 1987; Martinson *et al.*, 1987; Shimada *et al.*, 1991) (fig. 3). Of these, DPCPX has the greatest affinity (K_i 1.5 nM) for A_1 receptors and the greatest A_1-subtype selectivity (A_2/A_1 affinity ratio 740), as shown in rat brain membranes (Bruns *et al.*, 1987; Lohse *et al.*, 1987). The human A_1 receptor has an approximately lower affinity for DPCPX (Libert *et al.*, 1992; Klotz *et al.*, 1998). A number of other 8-substituted xanthines, including (±)-8-(3-oxocyclopentyl)-1,3-dipropylxanthine (KFM 19) and KW-3902 (8-noradamant-3-yl-1,3-dipropylxanthine), have been shown to be selective antagonists at A_1 receptors (see Williams, 1989; Jacobson *et al.*, 1992b). The alkylxanthine 1,3-dipropyl-8-[2-(5,6-epoxy)norbornyl]xanthine (ENX) is a potent (K_B 3.6 nM) and selective antagonist at A_1 receptors in the guinea- pig heart and brain and in DDT_1 MF-2 cells, with 400-fold greater affinity of binding versus A_{2A} receptors in guinea-pig brain (Belardinelli *et al.*, 1995b).

Several classes of non-xanthine antagonists have been described, some showing reasonable affinity and selectivity for the A_1 receptor (see Jacobson *et al.*, 1992b; Daly *et al.*, 1993). Some of the more active of these are the tricyclic non-xanthine antagonists, including the triazoloquinazolines (Francis *et al.*, 1988), the triazoloquinoxalines (Trivedi and Bruns, 1988; Sarges *et al.*, 1990), and the imidazoquinolines (Van Galen *et al.*, 1991).

The adenine derivative 1,3-dipropyl-8-[2,(5,6-epoxy)norbornyl]xanthine (N 0861) is reasonably selective (10- to 47-fold versus A_{2A} receptors) and potent at A_1 receptors in a number of tissues (May *et al.*, 1991; Martin *et al.*, 1993a; Belardinelli *et al.*, 1995b). This compound has been superceded by the S-enantiomer 12 (CVT-124) with nanomolar selectivity and 1800- and 2400-fold selectivity at rat and cloned human A_1 receptors, respectively (Pfister *et al.*, 1997), and by 8-(N-methylisopropyl)amino-N^6-(5′-endohydroxy-endonorbornyl-)9-methyl adenine (WRC 0571) with 62-fold selectivity versus the A_{2A} receptor and 4670-selectivity versus the A_3 receptor (Martin *et al.*, 1996).

(+)-(R)-[(E)-3-(2-phenylpyrazolo[1,5-α]pyridin-3-yl)acryloyl]-2-piperidine ethanol, FK 453, has been reported to be a potent and selective A_1 receptor antagonist with IC_{50} values of approximately 17 nM at rat cortical A_1 receptors and 11 μM at striatal A_2 receptors (Terai *et al.*, 1995). Chiral pyrolo[2,3-d]pyrimidine and pyrimido[4,5-b]indole derivatives have been shown to be potent and highly stereoselective A_1 adenosine receptor antagonists (Müller *et al.*, 1996a).

G. Distribution and Biological Effects

A_1 receptors are widely distributed in most species and mediate diverse biological effects. There is considerable literature in this area. Thus, this section is intended to give an indication of the ubiquity and diversity of actions mediated by adenosine at A_1 receptors, rather

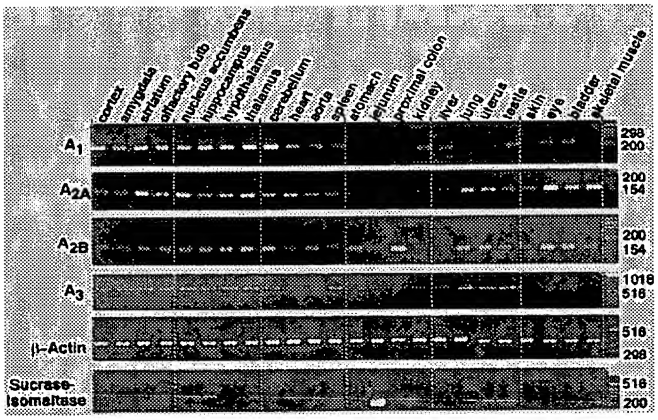


FIG. 4. Tissue distribution of adenosine receptor mRNA expression as examined by RT-PCR. Sizes of PCR products are given in base pairs. (From Dixon *et al.*, 1996, *Br J Pharmacol* **118**:1461–1468; with permission from McMillan Press Limited.)

than to provide a comprehensive account of A_1 receptor distribution and effects.

A_1 receptors are particularly ubiquitous within the central nervous system (CNS), with high levels being expressed in the cerebral cortex, hippocampus, cerebellum, thalamus, brain stem, and spinal cord (Reppert *et al.*, 1991; Dixon *et al.*, 1996) (fig. 4). Immunohistochemical analysis using polyclonal antisera generated against rat and human A_1 adenosine receptors has identified different labeling densities of individual cells and their processes in selected regions of the brain (Rivkees *et al.*, 1995b). A_1 receptor mRNA is widely distributed in peripheral tissues having been localized in vas deferens, testis, white adipose tissue, stomach, spleen, pituitary, adrenal, heart, aorta, liver, eye, and bladder (Reppert *et al.*, 1991; Dixon *et al.*, 1996). Only very low levels of A_1 mRNA are present in lung, kidney, and small intestine (Reppert *et al.*, 1991; Stehle *et al.*, 1992; Dixon *et al.*, 1996) (fig. 4).

It is now well established that adenosine is released from biological tissues during hypoxia and ischemic conditions. One of its effects is to reduce neuronal activity and thereby oxygen consumption; thus it acts as a neuroprotective agent. A significant part of these effects seem to be mediated by the A_1 receptor. A_1 receptors are located pre and postsynaptically on cell bodies, and on axons, where they mediate inhibition of neurotransmission by decreasing transmitter release, hyperpolarizing neuronal membranes, reducing excitability and firing rate, and altering axonal transmission. Adenosine can also exert behavioral effects: adenosine actions at A_1 receptors have been implicated in sedative, anticonvulsant, anxiolytic, and locomotor depressant effects (Nikodijevic *et al.*, 1991; Stone, 1991; Jain *et al.*, 1995; Malhotra and Gupta, 1997). Conversely, xanthine antagonists such as caffeine and theophylline have central stimulatory properties ascribed, at least in part, to inhibition of endogenous adenosine, although inhibition of cyclic nucleotide phosphodiesterases may contribute to this effect.

A_1 receptors mediate cardiac depression through negative chronotropic, dromotropic, and inotropic effects (see Olsson and Pearson, 1990). Slowing of the heart rate occurs via A_1 receptors on sinoatrial and atrioventricular nodes causing bradycardia and heart block, respectively, while the inotropic effects include a decrease in atrial contractility and action potential duration (Olsson and Pearson, 1990). This aspect of A_1 receptor-mediated effects has found application in the clinical use of adenosine to treat supraventricular tachycardia, and in the use of adenosine receptor antagonists in the treatment of bradyarrhythmias.

In the kidney, activation of A_1 receptors mediates diverse effects including vasoconstriction (principally of the afferent arteriole), a decrease in glomerular filtration rate, mesangial cell contraction, inhibition of renin secretion, and inhibition of neurotransmitter release (Olivera *et al.*, 1989; Agmon *et al.*, 1993; Barrett and Droppleman, 1993; Munger and Jackson, 1994). Intravenous and intra-aortic administration of adenosine in rats decrease water and sodium excretion via A_1 receptors, while selective antagonism of A_1 receptors causes diuresis and natriuresis (see Mizumoto *et al.*, 1993; Van Beuren *et al.*, 1993). Intrarenal administration of adenosine, but not of the A_{2A} selective agonist CGS 21680, in dogs also decreases water and sodium excretion (Levens *et al.*, 1991a,b). Furthermore, A_1 receptors increase transepithelial resistance and reduce Na^+ uptake in inner medullary collecting duct cells in culture (Yagil *et al.*, 1994). On the other hand, intrarenal administration of adenosine and the A_1-selective agonist CHA in rats has been shown to induce marked diuresis and natriuresis which can be inhibited by the A_1-selective antagonist DPCPX (Yagil, 1994).

Direct effects on blood vessel tone via adenosine actions on A_1 receptors are rare. A more significant role of A_1 receptors with regard to regulation of blood vessel tone appears to be prejunctional modulation of neurotransmitter release. Prejunctional inhibition of neurotransmission via A_1 receptors on perivascular sympathetic (Gonçalves and Queiroz, 1996) and capsaicin-sensitive sensory afferents (Rubino *et al.*, 1993) has been shown. However, A_1 receptors have been observed to mediate relaxation of porcine coronary artery (Merkel *et al.*, 1992), and contraction of guinea-pig aorta (Stoggall and Shaw, 1990) and pulmonary artery (Szentmiklósi *et al.*, 1995). A_1 receptors have also been reported to mediate contraction of rat isolated spleen (Fozard and Milavec-Krizman, 1993) and rat vas deferens (Hourani and Jones, 1994), as well as bronchoconstriction and bronchial hyperresponsiveness (Ali *et al.*, 1994a, 1994b; Pauwels and Joos, 1995; el-Hashim *et al.*, 1996). Diverse A_1-mediated effects in the gut have been described, including inhibition of peristalsis of rat jejunum (Hancock and Coupar, 1995b), relaxation of longitudinal muscle of

rat duodenum (Nicholls *et al.*, 1992, 1996), and contraction of rat colonic muscularis mucosa (Bailey *et al.*, 1992; Reeves *et al.*, 1993). Interestingly, adenosine mediates contraction of guinea-pig myometrial smooth muscle via A_1 receptors that in non-pregnant animals are coupled to the formation of IP_3, but in pregnant animals are coupled both to IP_3 and negatively to adenylate cyclase (Schiemann and Buxton, 1991; Schiemann *et al.*, 1991a,b).

Selective inhibition of the synthesis of A_1 receptors with antisense oligonucleotides confirmed that these receptors are involved in an animal model of asthma (Nyce and Metzger, 1997). There was a marked reduction in the number of A_1 receptors in the lung and attenuation of airway constriction to adenosine, histamine, and dust-mite allergen (Nyce and Metzger, 1997). Although the site of action remains to be determined, selective antagonism of A_1 receptors offers a possible new approach in asthma therapy.

A_1 receptors on bovine pulmonary artery endothelial cells have been shown to mediate Cl^- efflux (Arima *et al.*, 1994). In human airway epithelial cells, A_1 receptors have been reported to mobilize intracellular Ca^{2+} and activate K^+ and Cl^- conductance (Rugolo *et al.*, 1993), while selective inhibition of A_1 receptors with DPCPX increases cAMP-activated Cl^- conductance (McCoy *et al.*, 1995).

A_1 adenosine receptors on rat cochleal membranes (Ramkumar *et al.*, 1994), astrocytes (Peakman and Hill, 1994), and epididymal spermatozoa (Minelli *et al.*, 1995) have been described. Release of Ca^{2+} from internal stores in perisynaptic glial cells of the frog neuromuscular junction via A_1 receptors has been described (Robitaille, 1995).

Adenosine acts via A_1 receptors and inhibition of cAMP to inhibit lipolysis and increase insulin sensitivity in adipose tissue (Londos *et al.*, 1985; Green, 1987). Abnormal A_1 receptor function in genetic obesity has been proposed, showing that lipolysis is less active and A_1 receptor signaling more active, which may be caused by changes in receptor phosphorylation, but also possibly by adenylate cyclase activity (LaNoue and Martin, 1994; Berkich *et al.*, 1995). In contrast, insulin sensitivity is decreased by activation of A_1 receptors in skeletal muscle (Challis *et al.*, 1992). A_1 receptors on pancreatic β cells mediate inhibition of insulin secretion (Hillaire-Buys *et al.*, 1989).

A_1 receptors have been widely reported to mediate the protective effects of adenosine in preconditioning and during ischemia or during reperfusion injury in the heart (Tsuchida *et al.*, 1993, 1994; Yao and Gross, 1993; Lee *et al.*, 1995; Lasley and Mentzer, 1995; Strickler *et al.*, 1996; Grover *et al.*, 1992; van Winkle *et al.*, 1994; Sakamoto *et al.*, 1995; Mizumura *et al.*, 1996; Stambaugh *et al.*, 1997), lung (Neely and Keith, 1995), and brain (Heurteaux *et al.*, 1995). Strong evidence for a protective role of A_1 adenosine receptors comes from studies with transgenic mice over expressing the A_1 receptor. Mice over expressing the A_1 receptor have been shown to have an increased myocardial resistance to ischemia (Matherne *et al.*, 1997). The mechanism involved is not yet clear; it may involve A_1 receptor activation of K_{ATP} channels as infarct size reduction after activation of A_1 receptors has been reported to be completely abolished by the blockade of K_{ATP} channels (Grover *et al.*, 1992; van Winkle *et al.*, 1994; Mizumura *et al.*, 1996). On the other hand, there seems to be a general consensus that PKC is involved in ischemic preconditioning, and activation of PKC was shown to be the critical factor involved in limitation of myocardial infarct size by A_1 receptors in anaesthetized rabbits (Sakamoto *et al.*, 1995). However, not all researchers are in agreement that adenosine is cardioprotective, or that A_1 receptors mediate ischemic preconditioning (Asimakis *et al.*, 1993; Ganote *et al.*, 1993; Hendrikx *et al.*, 1993; Lasley *et al.*, 1993; Liu *et al.*, 1994). In addition, a protective role for adenosine A_3 receptors has been suggested (see Section VI.G.).

Reperfusion of ischemic tissue results in locally increased permeability and pulmonary edema that is associated with neutrophil accumulation in the microvasculature; neutrophil-endothelial cell interactions are known to be a prerequisite for the associated microvascular injury. Paradoxically, given the protective role of A_1 receptors in ischemia-reperfusion injury, adenosine contributes to inflammatory reactions via effects on neutrophil and/or endothelial A_1 receptors. This is done by augmenting responses to microbial stimuli, promoting chemotaxis, adhesion to endothelium, phagocytosis, and release of reactive oxygen intermediates (Cronstein *et al.*, 1990; Cronstein, 1994; Zahler *et al.*, 1994; Bullough *et al.*, 1995; Felsch *et al.*, 1995). It is possible that the local concentration of adenosine is crucial in determining which type of response predominates. A concentration-dependent dual protective-destructive role has also been described for the A_3 adenosine receptor, but what is even more intriguing is that it involves high and low levels of activation of A_3 receptors on the same cell (in both HL-60 and U 937 cells) (Yao *et al.*, 1997).

A_1 adenosine receptors have been implicated in modulation of nociception in the spinal cord (Reeve and Dickenson, 1995) and in the periphery (Karlsten *et al.*, 1992; Ocana and Baeyens, 1994). This may involve inhibition of sensory neurotransmitter release, because A_1 receptors have been shown to mediate inhibition of calcitonin gene-related peptide (CGRP) release from capsaicin-sensitive sensory neurons in the spinal cord (Santicoli *et al.*, 1993) and in the periphery (Rubino *et al.*, 1993), as well as inhibit GABA currents in dorsal root ganglion neurons (Hu and Li, 1997). Analgesic effects of caffeine have also been described. These effects have been attributed to caffeine's effects on supraspinal A_1 receptors because caffeine's effect is mimicked by the A_1-selective agonist 8-cyclopentyltheophylline (CPT);

spinally or peripherally administered caffeine lacks antinociceptive effects (Sawynok and Reid, 1996).

Synergistic interactions between A_1 adenosine receptors and receptors coupled to a different class of G protein, typically pertussis toxin insensitive $G_{q/11}$ proteins, have been described, whereby coactivation of the receptors results in an augmented increase in effectors/second-messengers derived from the $G_{q/11}$ protein coupled pathway. The intracellular mechanisms underlying this potentiation are not well understood and have been suggested variously to involve intra- and extracellular calcium, second-messengers, and G_i protein $\beta\gamma$ subunits. Early evidence for this kind of interaction came with the observation that adenosine enhances α_1-adrenoceptor-induced accumulation of cAMP in rat vas deferens (Häggblad and Fredholm, 1987). Synergistic interactions have since been shown in DDT_1 MF-2 cells for A_1 receptors and ATP receptors (Gerwins and Fredholm, 1992a), histamine H_1 receptors (Dickenson and Hill, 1994), and bradykinin receptors (Gerwins and Fredholm, 1992b). A_1 receptors transfected into CHO cells act synergistically with receptors for thrombin (Dickenson and Hill, 1997), cholecystokinin A (Dickenson and Hill, 1996), and ATP (Megson *et al.*, 1995). A_1 receptors in astrocytes interact synergistically with histamine H_1 receptors (Peakman and Hill, 1995) and glutamate receptors (Ogata *et al.*, 1994) to raise levels of $[Ca^{2+}]_i$. Synergistic interactions between A_1 and α_1-adrenoceptor mediated increases in inositol phosphate accumulation has been shown in mouse striatal astrocytes (el-Etr *et al.*, 1992a,b; Marin *et al.*, 1993). In hippocampal neurons, positive interactions have been described between adenosine A_1 and $GABA_A$ receptors (Akhondzadeh and Stone, 1994), as well as negative interactions between A_1 and metabotropic glutamate receptors (de Mendonça and Ribeiro, 1997). Cross-talk between A_1 and other receptors is clearly widespread; its physiological significance is an important area for future research.

IV. A_{2A} Receptor

A. Cloned A_{2A} Receptors

The A_{2A} receptor has been cloned from several species (table 3) and has a characteristic pharmacological profile in transfected cells consistent with that of the endogenous receptor. The first cloned adenosine receptor, RDC8, cloned from a canine thyroid cDNA library (Libert *et al.*, 1989), was subsequently identified as an A_{2A} receptor based on the binding of [^{3}H]NECA and [^{3}H]CGS 21680, and by activation of adenylate cyclase in cells transfected with the receptor (Maenhaut *et al.*, 1990). The exogenous A_{2A} receptor was shown to have a tissue distribution similar to endogenous A_{2A} binding sites in brain, that is, limited to the striatum, nucleus accumbens and olfactory tubercule (Schiffmann *et al.*, 1990). Subsequently, A_{2A} receptors were cloned from rat brain (Chern *et al.*, 1992; Fink *et al.*, 1992), human hippocampus (Furlong *et al.*, 1992), and guinea-pig brain (Meng *et al.*, 1994b). Both A_{2A} and A_{2B} receptors have been cloned from mouse bone marrow-derived mast cells (Marquardt *et al.*, 1994). The gene for the A_{2A} receptor has been mapped to human chromosome 22 (MacCollin *et al.*, 1994; Peterfreund *et al.*, 1996) with reported chromosomal localizations of 22q11.2 (Le *et al.*, 1996) and 22q11.2-q13.1 (Libert *et al.*, 1994).

In common with the other adenosine receptor subtypes, there is significant interspecies differences in the amino acid sequences of cloned A_{2A} receptors; for example, between rat and human A_{2A} receptors there is approximately 84% amino acid homology (Chern *et al.*, 1992; Fink *et al.*, 1992; Furlong *et al.*, 1992; Linden, 1994), and between rat and dog A_{2A} receptors 82% homology (Chern *et al.*, 1992; Fink *et al.*, 1992).

The significantly greater molecular weight of the A_{2A} receptor (45 kDa) compared with the other adenosine receptor subtypes (36 to 37 kDa) can largely be attributed to its substantially longer carboxy terminal domain. This region is not involved in tight coupling to G_s proteins because this is a function predominantly of the N-terminal segment of the third intracellular loop (Olah, 1997). A truncated mutant of the canine A_{2A} adenosine receptor was used to show that neither the long carboxy-terminus nor the glycosidic moieties are required for ligand binding (Piersen *et al.*, 1994). Site-directed mutagenesis of the human A_{2A} adenosine receptor has been used to identify the various residues involved in agonist and antagonist binding (Kim *et al.*, 1995; Ijzerman *et al.*, 1996).

B. Signal Transduction Mechanisms

The most commonly recognized signal transduction mechanism for A_{2A} receptors is activation of adenylate cyclase. This implies coupling with the G protein G_s, although other G proteins may also be involved. *Vibrio cholerae* (cholera toxin) ADP-ribosylates the α-subunit of G_s family members, inhibiting the intrinsic GTPase activity of $G_{\alpha s}$ and thus has been useful in characterizing members of this family. Coupling of the A_{2A} receptor to its G protein is tight (see Palmer and Stiles, 1995). Hence, there is only slow dissociation of agonist from the receptor and stabilization of the receptor-G protein complex.

cAMP-independent signaling has been suggested for A_{2A} receptors on striatal GABA nerve terminals (Kirk and Richardson, 1995) and striatal cholinergic nerve terminals (Gubitz *et al.*, 1996). In striatal nerve terminals, A_{2A} receptors are suggested to mediate dual signaling via P- and N-type Ca^{2+} channels linked to G_s/adenylate cyclase/PKA and cholera toxin-insensitive G protein/PKC, respectively (Gubitz *et al.*, 1996). It has been suggested that A_{2A} receptor-mediated inhibition of superoxide anion generation in neutrophils may be mediated via cAMP-independent activation of a serine/threonine protein phosphatase (Revan *et al.*, 1996).

A_{2A} receptor-mediated facilitation of synaptic transmission and transmitter release seems to occur through potentiation of presynaptic P-type Ca^{2+} channels, and probably involves adenylate cyclase and activation of a cAMP-dependent protein kinase (Mogul *et al.*, 1993; Correia-de-Sá and Ribeiro, 1994a; Umemiya and Berger, 1994; Gubitz *et al.*, 1996).

K_{ATP} channels are suggested to be involved in coronary vasodilatation mediated by A_2 receptors in the dog (Akatsuka *et al.*, 1994). Activation of K_{ATP} channels by A_2 receptors in arterial myocytes is suggested to involve a cAMP-dependent protein kinase (Kleppisch and Nelson, 1995).

C. Desensitization

Desensitization of A_{2A} receptors has been reported, which may be more rapid, similar to, or less rapid than that of A_1 receptors. In DDT_1 MF-2 cells, the $t_{1/2}$ for desensitization of A_{2A} receptors (45 min) is more rapid than that for A_1 receptors, and in contrast to A_1 receptors, there is no change in A_{2A} receptor number or affinity (Ramkumar *et al.*, 1991). A_{2A} receptor desensitization after exposure to A_2- or A_{2A}-selective agonists for up to several minutes to 4h has been observed in a number of tissues including porcine coronary artery (Makujina and Mustafa, 1993), rat aortic vascular smooth muscle cells (Anand-Srivastava *et al.*, 1989), DDT_1 MF-2 smooth muscle cells (Ramkumar *et al.*, 1991), rat pheochromocytoma PC12 cells (Chern *et al.*, 1993), and in canine A_{2A} receptors expressed in CHO cells (Palmer *et al.*, 1994). On the other hand, guinea-pig coronary artery A_{2A} receptors do not desensitize after more than 2h exposure to 2-[(2-aminoethylamino) carbonylethylphenylethylamino]-5′-N-ethylcarboxamido adenosine (APEC) or 1,4-phenylene-diisothiocyanate, 4-isothiocyanatophenyl aminothiocarbonyl-APEC (DITC-APEC) (Niiya *et al.*, 1993). Furthermore, A_{2A} receptors seem to be relatively resistant compared with A_1 receptors to desensitization in rat brain slices (Abbracchio *et al.*, 1992) and in spontaneously hypertensive rats after chronic treatment with A_1 and A_2 selective agonists in vivo (Casati *et al.*, 1994). In rat striatum slices, A_2 receptors do not desensitize following exposure to NECA for up to 1h, whereas A_1 receptors desensitize rapidly (Abbracchio *et al.*, 1992).

The mechanism underlying desensitization of A_{2A} receptors has been studied in some detail in transfected CHO cells, where it has been shown that exposure to agonist causes rapid desensitization and phosphorylation (Palmer *et al.*, 1994; Palmer and Stiles, 1997b). The threonine 298 residue of the carboxy terminal of the A_{2A} receptor seems to be essential for agonist-stimulated rapid receptor phosphorylation and short-term, but not long-term, desensitization (Palmer and Stiles, 1997a). The majority of the C terminal seems not to be involved in desensitization, because desensitization of a truncated mutant lacking the majority of the A_{2A} carboxyl-terminal (the last 95 residues) is unchanged (Palmer and Stiles, 1997a). Evidence that desensitization may involve GRKs, implying uncoupling of the receptor-G protein complexes, has been provided by a study in NG108–15 mouse neuroblastoma × rat glioma cells mutants overexpressing GRK2, where the rate of desensitization of endogenous A_{2A} and A_{2B} receptors was markedly slowed (Mundell *et al.*, 1997). This effect was selective in that agonist-induced desensitization of secretin and IP-prostanoid receptor stimulated adenylate cyclase were not affected by dominant negative mutant GRK2 overexpression (Mundell *et al.*, 1997). Receptor sequestration, whereby a receptor translocates to a "light membrane" fraction, has been described for A_{2A} receptors expressed in CHO cells, but this seems to be involved in the recovery of the response of the receptor rather than in desensitization (Palmer *et al.*, 1994).

Studies of long-term desensitization of endogenous A_{2A} receptors in rat pheochromocytoma PC12 cells showed that whereas a 30 min exposure of A_{2A} receptors to CGS 21680 is associated with inhibition of adenylate cyclase activity, long-term agonist exposure (12–20h) is associated additionally with down regulation of G_s α proteins and activation of phosphodiesterase (Chern *et al.*, 1993). Long-term (24h) exposure to agonist may additionally lead to down-regulation of receptor number and up-regulation of inhibitory G proteins (Palmer *et al.*, 1994; Palmer and Stiles, 1997a). Approximately 2 weeks of continuous infusion of either NECA or CGS 21680 causes a decrease in the number of A_{2A} receptor binding sites in rat striatum (Porter *et al.*, 1988; Webb *et al.*, 1993a). A calcium-independent PKC isoenzyme seems to be involved in phosphorylation and inhibition of adenylate cyclase type VI activity after prolonged stimulation and desensitization of the A_{2A} receptor, at least in rat pheochromocytoma PC12 cells (Lai *et al.*, 1997), providing an additional mechanism by which to regulate A_{2A} receptor signal transduction.

D. Sensitization / Up-Regulation

Striatal A_{2A} adenosine receptors in rats and mice are up-regulated after chronic caffeine ingestion (Hawkins *et al.*, 1988; Traversa *et al.*, 1994). A_{2A} receptors seem to be less prone to up-regulation after chronic blockade with non-selective antagonists than are A_1 receptors (Lupica *et al.*, 1991a; Johansson *et al.*, 1993a).

E. Agonists

A_{2A} receptors do not generally bind N^6-substituted adenosine derivatives and show a preference for derivatives with modifications of the 2nd position of the adenine ring; bulky substituents in this position can selectively enhance A_{2A} receptor affinity (Jacobson *et al.*, 1992b; Cristalli *et al.*, 1994; Siddiqi *et al.*, 1995). Several synthetic A_{2A}-selective agonists are modeled according to this structural modification. It should be noted that the agonist studies detailed below have been carried out in species other than humans, and that the human A_{2A}

receptor has a comparatively lower affinity of binding for CGS 21680 and other adenosine receptor agonists (Dionisotti *et al.*, 1997; Klotz *et al.*, 1998).

The C2-substituted NECA derivative, CGS 21680, is 140-fold selective for the A_{2A} versus the A_1 receptor (Hutchison *et al.*, 1990) (fig. 2). CGS 21680 has only very low affinity at the A_{2B} receptor, and thus has been used extensively to discriminate between A_{2A} and A_{2B} subtypes (Jarvis *et al.*, 1989; Lupica *et al.*, 1990). [^{3}H]CGS 21680 has been reported to bind in rat cortex and hippocampus to adenosine binding sites different to the classic striatal A_{2A} receptors, which does not seem to be caused by high and low affinity states of the same A_{2A} receptor, or to binding at A_3 or A_4 receptors (Johansson *et al.*, 1993b; Cunha *et al.*, 1996; Lindström *et al.*, 1996). Amine derivatives of CGS 21680, namely APEC (fig. 2), DITC-APEC and 2-[4-(2-([4-aminophenyl]methylcarbonyl)-ethyl)-phenyl]ethylamino-5′-N-ethylcarboxamidoadenosine (PAPA-APEC), are A_{2A}-selective agonists (Barrington *et al.*, 1989; Ramkumar *et al.*, 1991; Jacobson *et al.*, 1992a; Niiya *et al.*, 1993). DITC-APEC binds covalently, causing irreversible activation of the A_{2A} receptor (Niiya *et al.*, 1993).

The C2-substituted adenosine derivative CV 1808 displays poor selectivity (approximately 5-fold) for the A_{2A} versus the A_1 receptor (Kawazoe *et al.*, 1980; Bruns *et al.*, 1986), but is a valuable precursor for the synthesis of more selective A_{2A} receptor agonists. N^6-(2(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl)-adenosine (DPMA) is a selective A_{2A} receptor agonist (Merkel *et al.*, 1992; Alexander *et al.*, 1994).

A series of 2-aralkynyl and 2-heteroalkynyl derivatives of NECA have been studied for their selectivity at the A_{2A} receptor (Cristalli *et al.*, 1995). Of these, the 4-formylphenylethynyl derivative shows affinity in the low nanomolar range and approximately 160-fold selectivity. 2-Hexyl-5′-N-ethylcarboxamidoadenosine (2HE-NECA) has been suggested to be selective at A_{2A} receptors with 60- and 160-fold selectivity in binding studies for A_{2A} versus A_1 receptors in rat and bovine brain, respectively (Monopoli *et al.*, 1994). Although NECA itself is approximately equipotent at A_1 and A_{2A} receptors, it can be useful in A_{2A} receptor characterization provided that A_1-selective ligands are shown not to have equivalent effects.

The 2-hydrazinoadenosine, WRC-0470 (2-cyclohexylmethylidenehydrazinoadenosine) has been shown to be a potent and selective A_{2A} agonist, with low nanomolar affinity at recombinant A_{2A} receptors transfected in mammalian cells and in functional assays in a variety of tissues (Martin *et al.*, 1997b).

F. Antagonists

Several antagonists selective for the A_{2A} receptor have been synthesized. 8-(3-chlorostyryl)caffeine (CSC) is a potent (K_i 54 nM) and selective A_{2A} antagonist in radioligand binding assays in rat brain (520-fold selective versus A_1 receptors), in reversing agonist effects on adenylate cyclase in PC12 cells (22-fold selective), and in blocking locomotor depression elicited by the A_{2A}-selective agonist APEC in vivo (Jacobson *et al.*, 1993a) (fig. 3). 1,3-dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthine (KF-17837) has been described as a potent and selective A_{2A} antagonist with 62-fold selectivity for A_{2A} over A_1 receptors in binding studies in rat brain, and 30-fold selectivity for the A_{2A} over the A_{2B} receptor in inhibition of cAMP accumulation (A_{2A} IC_{50} = 53 nM; A_{2B} IC_{50} = 1500 nM) (Shimada *et al.*, 1992; Kanda *et al.*, 1994; Nonaka *et al.*, 1994). DMPX (3,7-dimethyl-1-propargylxanthine) derivatives have been shown to be potent and selective A_{2A} antagonists; 8-(m-bromostyryl)-DMPX has a K_i value of 8.2 nM and is 146-fold selective versus A_1 receptors (Müller *et al.*, 1996b).

ZM 241385, (4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo [2,3-*a*] [1,3,5]triazin-5-yl amino]ethyl)phenol) is a potent and selective non-xanthine A_{2A} adenosine receptor antagonist (Poucher *et al.*, 1995) (fig. 3). It has high affinity for the A_{2A} receptor (pA_2 value approximately 9), is 1000- and 91-fold selective versus A_1 and A_{2B} receptors, respectively, and has virtually no effects at A_3 receptors (Poucher *et al.*, 1995).

[^{3}H]SCH 58261 ([^{3}H-5-amino-7-(2-phenylethyl)-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c] pyrimidine) is a novel potent and selective A_{2A} antagonist radioligand which binds with low nanomolar affinity to A_{2A} receptors in human platelet and rat striatal membranes, and at A_{2A} receptors transfected into CHO cells (Zocchi *et al.*, 1996; Dionisotti *et al.*, 1997). The analog SCH 63390 (5-amino-7-(3-phenylpropyl)-2-(2-furyl)pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine) has similar potency at A_{2A} receptors, but greater selectivity (210-fold) (Baraldi *et al.*, 1996).

G. Distribution and Biological Effects

A_{2A} receptors have a wide-ranging but restricted distribution that includes immune tissues, platelets, the CNS, and vascular smooth muscle and endothelium. Functional studies concerned with A_{2A} receptors in isolated cells and tissues, in the central and peripheral nervous systems, and in isolated blood vessels and vascular beds, are listed in tables 4, 5 and 6, and illustrate the wide distribution and diverse biological effects mediated by this receptor.

Within the brain, the highest levels of A_{2A} receptors are in the striatum, nucleus accumbens, and olfactory tubercle (regions which are rich in dopamine) (Ongini and Fredholm, 1996). Low levels of A_{2A} receptor also seem to be expressed in most other brain regions, although for striatal cholinergic neurons this is controversial (Dixon *et al.*, 1996; Peterfreund *et al.*, 1996; Jin and Fredholm, 1997; Svenningsson *et al.*, 1997). Striatal neurons express A_{2A} receptors in close association with dopamine D_2 receptors and specific negative interactions have been described (Férre *et al.*, 1991, 1992, 1997;

TABLE 4
Distribution and effects mediated by endogenously expressed A_2 adenosine receptors

Tissue	Subtype	Effects	Reference
Astrocytes	A_2	Reactive astroglyosis	Hindley *et al.*, 1994
Astrocytes, type 1	A_{2B}	—	Peakman and Hill, 1994, 1996
Astrocytes, type 2	A_{2A}, A_{2B}	—	Peakman and Hill, 1996
Astroglioma cell line D384	A_{2B}	—	Altiok *et al.*, 1992; Fredholm and Altiok, 1994
Astrocytoma cell line U373	A_{2B}	↑ Interleukin 6	Fiebich *et al.*, 1996
Neutrophils	A_{2A}	↓ Apoptosis	Zhang *et al.*, 1996; Walker *et al.*, 1996
	A_{2A}	↓ Oxygen radical generation, phagocytosis and adhesion	Cronstein *et al.*, 1990, 1992; Salmon and Cronstein, 1990; Gurden *et al.*, 1993; Cronstein, 1994; Bullough *et al.*, 1995; Felsch *et al.*, 1995
Jurkat cells (human T-cell line)	A_{2A}, A_{2B}	—	Nonaka *et al.*, 1994; van der Ploeg *et al.*, 1996
Mast cells (mouse)	A_{2A}, A_{2B}	—	Marquardt *et al.*, 1994
Mastocytoma cells (canine)	A_{2B}	Degranulation	Auchampach *et al.*, 1997a
HMC-1 (human mast cell line)	A_{2A}, A_{2B}	Interleukin-8 secretion by A_{2B}	Feoktistov and Biaggioni, 1995
Fibroblasts	A_{2B}	—	Bruns *et al.*, 1986; Brackett and Daly, 1994
Platelets	A_{2A}	↓ Aggregation	Huttemann *et al.*, 1984; Gurden *et al.*, 1993; Monopoli *et al.*, 1994; Cristalli *et al.*, 1995
Chromaffin cells	A_{2B}	↓ DMPP-evoked catecholamine release	Casado *et al.*, 1992; Mateo *et al.*, 1995
Pheochromocytoma PC12 cells	A_2	↑ ATP-evoked dopamine release	Koizumi *et al.*, 1994
Pheochromocytoma PC12 cells	A_{2A}, A_{2B}	—	Hide *et al.*, 1992; Chern *et al.*, 1993; Nonaka *et al.*, 1994; van der Ploeg *et al.*, 1996
Pineal gland	A_{2B}	—	Gharib *et al.*, 1992
Retinal membranes	A_{2A}, A_{2B}	—	Blazynski and McIntosh, 1993
Retinal pigment epithelial cells	A_{2B}	—	Blazynski, 1993
Outer rod segments	A_{2A}	—	McIntosh and Blazynski, 1994
Airways	A_{2B}	Bronchoconstriction	Pauwels and Joos, 1995
Trachea	$A_{2(B)}$	Relaxation	Losinski and Alexander, 1995
Taenia coli	A_2	Relaxation	Burnstock *et al.*, 1984
Duodenum; longitudinal muscle	A_{2B}	Relaxation	Nicholls *et al.*, 1992
Duodenum; muscularis mucosae	A_{2B}	Contraction	Nicholls *et al.*, 1996
Colon	A_{2B}	—	Stehle *et al.*, 1992
Caecum	A_{2B}	—	Stehle *et al.*, 1992
Intestine	A_{2B}	↓ Secretion	Hancock and Coupar, 1995a
Intestinal epithelia	A_{2B}	↑ Cl^- secretion	Strohmeier *et al.*, 1995
Parietal cells	A_2	↑ Gastric acid secretion	Ainz *et al.*, 1993
Liver	A_2	↑ Glycogenolysis	Buxton *et al.*, 1987
Hepatocytes	A_2	↑ Glycogenolysis	Stanley *et al.*, 1987
Kidney	A_2	Erythropoietin production	Nakashima *et al.*, 1993
Kidney	A_2	↑ Renin release	Churchill and Churchill, 1985; Churchill and Bidani, 1987
Glomeruli	A_2	—	Freissmuth *et al.*, 1987
Pancreatic A cells	A_2	↑ Glucagon secretion	Chapal *et al.*, 1985
Bladder	A_{2B}	—	Nicholls *et al.*, 1992; Stehle *et al.*, 1992
Sperm	A_2	↑ Motility	Shen *et al.*, 1993

Fink *et al.*, 1992; Schiffmann and Vanderhaeghen, 1993). Outside the brain, the most abundant expression of human A_{2A} mRNA is in immune tissues, eye and skeletal muscle; heart, lung, bladder, and uterus also show strong expression, with less abundant expression in small intestine, kidney, spleen, stomach, testis, skin, kidney, and liver (Dixon *et al.*, 1996; Peterfreund *et al.*, 1996).

A_{2A} receptors in the CNS and particularly in the peripheral nervous system (PNS) generally facilitate neurotransmitter release (table 5).

The negative interactions that have been observed between A_{2A} and dopamine D_2 receptors involve a reduced affinity of agonist binding to dopamine D_2 receptors upon stimulation of A_{2A} receptors in rat striatal membranes (Ferré *et al.*, 1991, 1992, 1997). This raises the possibility of using A_{2A} receptor antagonists as a novel therapeutic approach in the treatment of Parkinsons disease, to reduce the profound disabling effects arising from degeneration of dopaminergic nigrostriatal neurons of the basal ganglia in this disease (Richardson *et al.*, 1997). Interactions are not observed between A_{2A} and D_2 receptors transfected into COS-7 cells; it was suggested that the receptors do not interact directly to influence agonist binding (Snaprud *et al.*, 1994). Interestingly, activation of A_{2A} receptors on rat striatal nerve terminals causes desensitization of coexpressed A_1 receptors by a mechanism which seems to involve PKC (Dixon *et al.*, 1997a). It is noteworthy that both D_2 dopamine and A_1 adenosine receptors couple to G_i proteins to cause inhibition of adenylate cyclase. Thus, with respect to the actions of adenosine at A_{2A} receptors, negative A_{2A}-A_1 and A_{2A}-D_2 interactions will shift the balance of intracellular signaling further toward stimulation of cAMP. Interactions between A_{2A} receptors and dopamine D_1 receptors, and receptors for CGRP, glutamate, and acetylcholine have also been reported (see Sebastião and Ribeiro, 1996). Negative interactions whereby activation of the A_{2A} receptor blocks the protective effects of preconditioning hypoxia, believed to be via A_1 and A_3 receptors, have been described (Strickler *et al.*, 1996).

TABLE 5
Functional distribution of endogenously expressed A_2 adenosine receptors in central and peripheral nervous systems

Location	Subtype	Effects	Reference
CNS			
Caudate-putamen synaptosomes	A_{2A}	↓ K^+-evoked GABA release	Kurokawa *et al.*, 1994
Cerebral cortex	$A_{2(A)}$	↓ Neuronal firing	Phillis, 1990; Lin and Phillis, 1991
Cerebral cortex	$A_{2(B)}$	↑ ACh- and K^+-evoked aspartate release	Phillis *et al.*, 1993a,b
Cerebral cortex	A_{2A}	↓ Ischemia-evoked GABA release	O'Regan *et al.*, 1992a
Cerebral cortex	A_{2A}	↓ Ischemia-evoked glutamate and aspartate release	O'Regan *et al.*, 1992b
Globus pallidus	A_{2A}	↑ Electrically evoked GABA release	Mayfield *et al.*, 1993
Globus pallidus synaptosomes	A_{2A}	↓ K^+-evoked GABA release	Kurokawa *et al.*, 1994
Hippocampus	A_{2A}	↑ Electrically evoked [^{14}C]ACh release	Jin and Fredholm, 1997
Hippocampus (CA3 region)	A_{2A}	↑ Electrically evoked [^{3}H]ACh release	Cunha *et al.*, 1994
Hippocampus (CA3 region)	A_2	↑ P-type calcium currents	Mogul *et al.*, 1993
Hippocampal synaptosomes	A_{2A}	↑ Veratridine-evoked [^{3}H]ACh release	Cunha *et al.*, 1995
Nucleus accumbens	A_{2A}	↓ Locomotor activity (baroreceptor ↓, chemoreceptor ↑)	Barraco *et al.*, 1993, 1994
Nucleus tractus solitarius	A_{2A}	Baroreflex control (hypotension, bradycardia)	Barraco *et al.*, 1993; Ergene *et al.*, 1994
	A_{2A}	↑ Electrically evoked [^{3}H]NA release	Barraco *et al.*, 1995
	A_{2A}	↓ K^+-evoked glutamate release	Castillo-Meléndez *et al.*, 1994
Striatum	A_{2A}	Catalepsy	Hauber and Munkle, 1995
Striatum	A_2	↑ Dopamine release	Zetterström and Fillenz, 1990
Striatum	A_{2A}	↑ ACh release	Brown *et al.*, 1990; Kurokawa *et al.*, 1994
Striatum	A_{2A}	↑ Veratridine-evoked [^{3}H]ACh release	Kirkpatrick and Richardson, 1993
Striatum	A_{2A}	↓ NMDA receptor conductance	Nörenberg *et al.*, 1997b
Striatal synaptosomes	A_{2A}	↓ K^+-evoked GABA release	Kirk and Richardson, 1995
Superior colliculus	A_{2A}	↑ Evoked potentials	Ishikawa *et al.*, 1997
Spinal cord	A_2	Antinociception	DeLander and Hopkins, 1987
PNS			
Motor nerves; phrenic nerve-hemidiaphragm	A_{2A}	↑ Electrically and CGRP-evoked [^{3}H]ACh release	Correia-de-Sá and Ribeiro, 1994a,b; Correia-de-Sá *et al.*, 1996
Myenteric neurones	A_{2A}	↑ Excitability	Christofi *et al.*, 1994
Airway sensory neurones	$A_{2(A)}$	↓ Capsaicin-evoked substance P release	Morimoto *et al.*, 1993
Vagal afferent neurones	A_{2A}	Depolarization	Castillo-Melendez *et al.*, 1994
Vas deferens neurones	A_{2A}	↑ Electrically evoked NA release	Gonçalves and Queiroz, 1993
Rat tail artery neurones	A_{2A}	↑ Electrically evoked NA release	Gonçalves and Queiroz, 1996

Behavioral effects of A_{2A} receptors are evidenced by A_{2A}-mediated cataleptic activity and antagonism of apomorphine-induced climbing (an animal model of schizophrenia) (Kanda *et al.*, 1994; Kafka and Corbett, 1996).

In the vasculature, A_{2A} receptors have been described on both the smooth muscle and endothelium, where they are associated with vasodilatation (table 6). There seems to be considerable variation in A_{2A} receptor expression between blood vessels, although it is possible that vessels unresponsive to A_{2A}-selective agonists do express the receptor but at very low levels, or that the receptor is not coupled to a functional response. This functional diversity is exemplified by the fact that A_{2A} receptors mediate relaxation of rat aorta and bovine coronary artery (Conti *et al.*, 1993), whereas in guinea-pig pulmonary artery (Szentmiklósi *et al.*, 1995) and rat mesenteric arterial bed (Rubino *et al.*, 1995), adenosine-mediated relaxation is mediated via the A_{2B} receptor, and relaxation via A_{2A} receptors is weak or non existent (fig. 5). Adenosine has a mitogenic effect on endothelial cells, which in human endothelial cells is mediated via the A_{2A} receptor and subsequent activation of mitogen-activated protein kinase (MAPK) (Sexl *et al.*, 1997). The mitogenic activation seems to be independent of G_s, G_i and typical PKC isoforms, but is associated with activation of $p21^{ras}$ (Sexl *et al.*, 1997).

An interesting development in this field is provided by a study of A_{2A} receptor knockout mice (Ledent *et al.*, 1997). These mice showed reduced exploratory activity. Caffeine, which normally stimulates locomotor activity, substantially depressed activity. The A_{2A} knockout mice also showed increased aggresiveness, hypoalgesia, an increase in blood pressure and heart rate, and an increase in platelet aggregation (Ledent *et al.*, 1997). It is satisfying that these findings are broadly consistent with those predicted from studies of the endogenous A_{2A} receptor in isolated cells and tissues, and in whole animals.

V. A_{2B} Receptor

A. Cloned A_{2B} Receptors

A_{2B} receptors have been cloned from human hippocampus (Pierce *et al.*, 1992), rat brain (Rivkees and Reppert, 1992; Stehle *et al.*, 1992), and mouse bone marrow-derived mast cells (Marquardt *et al.*, 1994) (table 3). The human A_{2B} adenosine receptor gene (ADORA2B) has been localized to chromosome 17p11.2-p12 (Townsend-Nicholson *et al.*, 1995b) and 17p12 (Jacobson *et al.*, 1995a). A human A_{2B} receptor pseudogene has been cloned and localized to chromosome 1q32 (Jacobson *et al.*, 1995a). Although the pseudogene is unable to encode a functional receptor, it is 79% identical with the functional A_{2B} receptor. Thus, it was noted that the existence of the transcript in tissues could lead to misinterpretation of in situ hybridization and northern blot analysis when probes are used to recognize sequences common to these receptors (Jacobson

TABLE 6
Functional distribution of endogenously expressed vascular A_2 adenosine receptors

Vessel and species	Receptor	Location	Reference
Aorta; guinea-pig	A_{2B}	EC, SM	Hargreaves *et al.*, 1991; Martin, 1992; Martin *et al.*, 1993b; Gurden *et al.*, 1993; Alexander *et al.*, 1994
Aorta; rabbit	A_{2A}	N.D.	Balwierczak *et al.*, 1991
Aorta; rat	A_{2A}, A_{2B}	EC, SM[a]	Conti *et al.*, 1993; Lewis *et al.*, 1994; Monopoli *et al.*, 1994; Prentice and Hourani, 1996
Aortic EC; human	A_{2A}, A_{2B}	EC	Iwamoto *et al.*, 1994
Aortic SM cells; rat	A_{2B}	SM	Dubey *et al.*, 1996
Coeliac artery; rabbit	A_{2A}	N.D.	Balwierczak *et al.*, 1991
Coronary artery; bovine	A_{2A}	N.D.	Conti *et al.*, 1993; Monopoli *et al.*, 1994
Coronary artery; canine	A_{2A}	N.D.	Balwierczak *et al.*, 1991; Gurden *et al.*, 1993
Coronary artery; human	A_{2A}	N.D.	Makujina *et al.*, 1992
Coronary artery; porcine	A_{2A}, $A_{2(B)}$	EC, SM	Balwierczak *et al.*, 1991; Abebe *et al.*, 1994; Monopoli *et al.*, 1994
Coronary artery EC; guinea-pig	A_{2A}	EC	Schiele and Schwabe, 1994
Coronary bed/vessels; guinea-pig	A_{2A}	EC, SM	Martin *et al.*, 1993b; Vials and Burnstock, 1993
Corpus cavernosum; rabbit	A_{2B}	EC, SM	Chiang *et al.*, 1994
DDT1 MF-2 cells (SM cells)	A_{2A}	SM	Ramkumar *et al.*, 1991
Hepatic arterial bed; rabbit	A_{2A}	N.D.	Mathie *et al.*, 1991a,b
Mammary artery; human	A_{2A}	N.D.	Makujina *et al.*, 1992
Mesenteric arterial bed; rat	A_{2A}	EC, SM	Hiley *et al.*, 1995
Mesenteric arterial bed; rat	A_{2B}	SM	Rubino *et al.*, 1995
Mesenteric artery; rabbit	A_{2A}	N.D.	Balwierczak *et al.*, 1991
Placental arterial bed; human	A_{2A}	N.D.	Read *et al.*, 1993
Pulmonary artery; guinea pig	A_{2B}	SM	Szentmiklósi *et al.*, 1995
Pulmonary arterial bed; rat	A_{2B}	SM	Haynes *et al.*, 1995
Pulmonary artery and vein; rabbit	A_2	EC, SM	Steinhorn *et al.*, 1994
Pulmonary arterial bed; rabbit	A_2	N.D.	Pearl, 1994
Renal artery; rat	A_{2B}	EC	Martin and Potts, 1994
Renal bed; rat	A_{2A}	SM	Levens *et al.*, 1991a,b; Agmon *et al.*, 1993
Saphenous vein; canine	A_{2B}	N.D.	Hargreaves *et al.*, 1991
Saphenous vein; human	A_{2A}	N.D.	Makujina *et al.*, 1992
Umbilical vein EC; human	A_{2A}	EC	Sobrevia *et al.*, 1997

EC, endothelium; SM, smooth muscle; N.D., not determined.
[a] A_{2A} adenosine receptor only.

et al., 1995a). As with the other adenosine receptor subtypes, there is considerable species differences in the sequence of the A_{2B} receptor; for example, 86% amino acid sequence homology between rat and human A_{2B} receptors (Stehle *et al.*, 1992; Pierce *et al.*, 1992; Linden, 1994).

B. Signal Transduction Mechanisms

A_{2B} receptor coupling to different signaling pathways has been reported, including activation of adenylate cyclase, G_q/G_{11}-mediated coupling to PLC and IP_3-dependent increase in $[Ca^{2+}]_i$ (in human mast cells) (Feoktistov and Biaggioni, 1995), and coupling to PLC when expressed in *Xenopus* oocytes (Yakel *et al.*, 1993).

C. Desensitization

The lack of A_{2B} receptor-selective agonists has undoubtedly contributed to the general lack of information on A_{2B} receptor desensitization. In rat PC12 cells, the A_{2B} response has been shown to be reduced in A_{2A}-desensitized cells, possibly through common inhibition of adenylate cyclase (Chern *et al.*, 1993). In mutant NG108–15 cells overexpressing GRK2, desensitization of endogenous A_{2B} receptors was markedly less than that in normal cells ($t_{1/2}$ 15–20 min), indicating that receptor phosphorylation and uncoupling from G proteins may be involved in desensitization of A_{2B} receptors (Mundell *et al.*, 1997). Although it is not yet clear whether there are inherent differences in the rates of desensitization of A_{2A} and A_{2B} receptors, the lower affinity of A_{2B} receptors for adenosine raises the possibility that they may still be fully operational, and thus may act as a backup for adenosine responses, when the higher affinity coexpressed A_{2A} receptors have been activated and desensitized.

D. Agonists and Antagonists

Despite intensive efforts in this area, there are no A_{2B}-selective agonists. Thus, at present, activation of adenylate cyclase in membranes and accumulation of cAMP in cells is used to characterize A_{2B} receptors, provided a lack of activity/binding of A_1-, A_{2A}-, and A_3-selective agonists is confirmed. As with A_{2A} receptors, A_{2B} receptors show a preference for adenosine derivatives with modifications of the C2 position of the adenine ring. NECA is currently the most potent agonist at A_{2B} receptors, having low micromolar affinity (Brackett and Daly, 1994; Alexander *et al.*, 1996; Klotz *et al.*, 1998), but is less useful in characterization of A_{2B} receptors in cells or tissues in which A_{2A} receptors are coexpressed because it is non-selective. 2-ClADO, N^6-(3-iodobenzyl)-5′-(N-methylcarbamoyl)adenosine (IB-MECA), and R-PIA are among the more potent of other conventional adenosine-receptor agonists that act also at A_{2B} receptors, but their affinity for the A_{2B} receptor is relatively low (EC_{50} values 9 to 11 μM) (Brackett and Daly, 1994; Klotz *et al.*, 1998).

Enprofylline blocks A_{2B} receptors in human mast cells HMC-1 (K_i 7 μM) and canine BR mastocytoma cells and

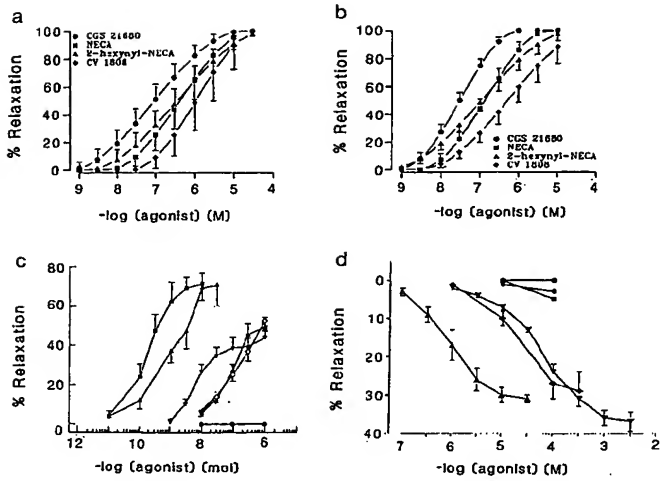


FIG. 5. Species variation in functional expression of vasodilator A_{2A} and A_{2B} receptors. Note that the agonist potencies suggest the presence of A_{2A} receptors in rat aorta (a) and bovine coronary artery (b), and A_{2B} receptors in rat mesenteric arterial bed, (c) and guinea-pig pulmonary arteries (d).

a., b. Mean dose-response curves for the vasorelaxant activity induced by some adenosine agonists in isolated rat aorta (a) and bovine coronary artery (b). Each response is expressed as the percentage of the maximum contraction induced by PGF2α (3 μM). *Vertical bars* represent 95% confidence limits. (From Conti *et al.*, 1993).

c. Dose-response curves showing vasodilator responses of the rat mesenteric vascular bed to ATP (▲), 2-meSATP (■), adenosine (◇), 2-CADO (★), NECA (▼), CPA (○) and CGS 21680 (●). Vasodilator response are shown as percent vasodilatation of the methoxamine sustained tone taken as 100% and are the mean of 4 to 7 preparations. Response are to bolus injections of drugs. Symbols show means ± SEM (From Rubino *et al.*, 1995, *Br J Pharmacol* **115**: 648–652; with permission from McMillam Press Limited).

d. Concentration-dependent relaxation of guinea pig pulmonary arteries by NECA (▲; n = 5), CADO (◆; n = 5), adenosine (▼; n = 16), CGS 21680 (■; n = 5), R-PIA (◆; n = 5) or CPA (●; n = 15). Relaxant responses are expressed as a percentage of the noradrenaline-contraction (mean ± SEM). (From Szentmiklósi *et al.*, 1995).

is inactive at A_1, A_{2A}, and A_3 receptors. It may, therefore, be a valuable starting compound from which to develop more potent selective A_{2B} receptor antagonists (Feoktistov and Biagionni, 1996). The non-xanthine alloxazine has been reported as having approximately 9-fold selectivity for the A_{2B} compared with the A_{2A} receptor (Brackett and Daly, 1994). XAC and CGS 15943 are antagonists with low nanomolar affinity at A_{2B} receptors, but are non-selective versus other subtypes of adenosine receptor (Alexander *et al.*, 1996; Klotz *et al.*, 1998).

E. Distribution and Biological Effects

A_{2B} receptors are found on practically every cell in most species; however, the number of receptors is small and relatively high concentrations of adenosine are generally needed to evoke a response. The sensitive technique of reverse transcription-polymerase chain reaction (RT-PCR) showed low levels of A_{2B} receptors in all rat brain regions tested (Dixon *et al.*, 1996). Northern blot analysis showed relatively high expression of A_{2B} receptors in the caecum, large intestine, and urinary bladder, with lower levels in the brain, spinal cord, lung, vas deferens, and pituitary (Stehle *et al.*, 1992). RT-PCR revealed the highest expression of A_{2B} receptors in the proximal colon, with lower levels in the eye, lung, uterus, and bladder; still lower levels in the aorta, stomach, testis, and skeletal muscle; and the lowest levels in the jejunum, kidney, heart, skin, spleen, and liver (Dixon *et al.*, 1996).

Selected distributions and biological effects mediated by A_{2B} receptors in isolated cells and tissues are listed in tables 4 and 6. Functional studies have identified A_{2B} receptors in airway smooth muscle, fibroblasts, glial cells, the gastrointestinal tract, and the vasculature. A_{2B} receptors have been cloned from, and immunolocalized on, mouse bone marrow-derived mast cells (Marquardt *et al.*, 1994), and shown to mediate degranulation of canine BR mastocytoma cells (Auchampach *et al.*, 1997a). They have also immunolocalized and been shown to activate human mast cells (Feoktistov and Biagionni, 1996). This implies a possible role in allergic and inflammatory disorders. The antiasthmatic effects of enprofylline, a potential A_{2B} receptor antagonist, are consistent with this hypothesis (Feoktistov and Biaggioni, 1996).

Vascular A_{2B} receptors identified by pharmacological and biochemical studies are listed in table 6, which shows that these receptors may couple to a functional response (vasodilatation) in both smooth muscle and endothelium. Interestingly, A_{2B} receptors seem to be important in mediating vasodilatation in some vessels, including the rat mesenteric arterial bed (Rubino *et al.*, 1995) and guinea-pig pulmonary arteries (Szentmiklósi *et al.*, 1995), but not in others where the A_{2A} subtype predominates (table 6, fig. 5). Rat aortic smooth muscle A_{2B} receptors have been implicated in inhibition of growth (Dubey *et al.*, 1996), identifying a possible long-term trophic role for these receptors.

VI. A_3 Receptor

A. Cloned A_3 Receptors

A_3, the fourth distinct adenosine receptor, was identified relatively late in the history of adenosine/P1 receptors with the cloning, expression, and functional characterization of a novel adenosine receptor from rat striatum (Zhou *et al.*, 1992). This was identical with a clone previously isolated from a rat testis cDNA library encoding a G protein-coupled receptor with greater than 40% sequence homology with canine A_1 and A_{2A} adenosine receptors, although its ligand had not then been identified (Meyerhof *et al.*, 1991). The recombinant striatal A_3 receptor does not resemble any other adenosine/P1 subtypes in agonist or antagonist binding; it binds ligands with a potency order of R-PIA = NECA > S-PIA and is coupled to inhibition of adenylate cyclase activity in a pertussis toxin-sensitive manner; it binds with high affinity to the radioligand N^6-2-(3-iodo-4-

aminophenyl)ethyladenosine but not to the A_{2A}-selective adenosine ligand [^{3}H]CGS 21680 or the alkylxanthine antagonists XAC, IBMX, or the A_1-selective antagonist DPCPX.

Homologs of the rat striatal A_3 receptor have been cloned from sheep pars tuberalis (pituitary tissue) (Linden *et al.*, 1993), human heart (Sajjadi and Firestein, 1993, and striatum (Salvatore *et al.*, 1993) (see also Linden, 1994) (table 3). Interspecies differences in A_3 receptor structure are large; the rat A_3 receptor shows only approximately 74% sequence homology with sheep and human A_3 receptors each, although there is 85% homology of sheep and human A_3 receptors. This is reflected in the very different pharmacological profiles of the species homologs, particularly with respect to antagonist binding, and this has caused considerable complications in the characterization of this receptor. The human A_3 receptor has been localized to chromosome 1 p13.3 (Monitto *et al.*, 1995).

The rat, but not the human, A_3 receptor transcript may be subject to extensive alternative splicing, further evidence of the profound interspecies differences involving the A_3 receptor. A splice variant of the rat A_3 receptor (A_{3i}), having a 17 amino acid insertion within the second intracellular loop, has been cloned and characterized (Sajjadi *et al.*, 1996). There was no evidence for alternative splicing of the human A_3 receptor transcript (Sajjadi *et al.*, 1996).

This A_3 receptor has taken precedence over the controversial A_3 receptor defined principally according to its pharmacological profile by Ribeiro and Sebastião (1986), which probably represents an A_1 receptor (Carruthers and Fozard, 1993; Ribeiro and Sebastião, 1994).

B. Signal Transduction Mechanisms

The A_3 receptor is G protein-linked, coupling to $G_i\alpha_2$-, $G_i\alpha_3$- and, to a lesser extent, to $G_{q/11}$ proteins (Palmer *et al.*, 1995b). In rat basophilic leukemia cells (RBL-2H3; a cultured mast cell line) (Ali *et al.*, 1990; Ramkumar *et al.*, 1993b) and in rat brain (Abbracchio *et al.*, 1995a), the A_3 receptor stimulates PLC and elevates IP_3 levels and intracellular Ca^{2+}. PKC has been suggested to be involved in A_3 receptor-mediated preconditioning in rabbit cardiomyocytes (Armstrong and Ganote, 1994). The A_3 receptor has also been shown to inhibit adenylate cyclase activity (Zhou *et al.*, 1992; Abbracchio *et al.*, 1995b).

C. Desensitization

Recombinant rat and human A_3 receptors have been shown to desensitize within minutes in response to agonist exposure; this is associated with uncoupling of the receptor-G protein complex, as indicated by a reduction in the number of high affinity binding sites (Palmer *et al.*, 1995a; Palmer *et al.*, 1997). Desensitization of the rat A_3 receptor is rapid (within a few minutes), homologous, and is associated with rapid phosphorylation by a G protein-coupled receptor kinase similar to, or identical with, GRK2 (Palmer *et al.*, 1995a; Palmer and Stiles, 1997b). Rapid, homologous functional desensitization of A_3 receptors has also been described in RBL-2H3 cells (Ali *et al.*, 1990; Ramkumar *et al.*, 1993b). A chimeric A_1-A_3 receptor constructed from an A_1 receptor (nondesensitizing under the conditions of the study) and the C-terminal domain of an A_3 receptor was expressed in CHO cells and shown to undergo rapid desensitization. This indicates that the C-terminal domain of the A_3 receptor is the site for phosphorylation by the G protein-coupled receptor kinases involved in desensitization (Palmer *et al.*, 1996).

The effects of long-term agonist exposure on interaction of the rat A_3 receptor with G proteins was assessed using a transfected CHO cell system (Palmer *et al.*, 1995b). Chronic exposure of A_3 receptors to the nonselective agonist NECA (for up to 24h) causes selective down-regulation of $G_i\alpha_3$- and β-subunits, without changing levels of $G_i\alpha_2$ or G_q-like proteins (Palmer *et al.*, 1995b).

D. Up-Regulation

In situ hybridization identified the A_3 receptor in mesenchymal cells and eosinophils within the lamina propria of the airways and the adventitia of blood vessels in the lung, as well as in peripheral eosinophils, but interestingly, not in mast cells (Walker *et al.*, 1997). It was found that the A_3 receptor transcript was greater in lung tissue from subjects with airway inflammation than in normal lung. This is consistent with the hypothesis that there is a distinct distribution of the A_3 receptor in inflammatory cells and that this is up-regulated in airway inflammation (Walker *et al.*, 1997).

E. Agonists

The main class of selective A_3 receptor agonists is the N^6-substituted adenosine-5′-uronamides. N^6-benzylNECA is potent (K_i 6.8 nM) and moderately selective (13- and 14-fold versus A_1 and A_{2A}) at rat A_3 receptors transfected into CHO cells (van Galen *et al.*, 1994). N^6-(3-iodobenzyl)-5′-(N-methylcarbamoyl)adenosine (IB-MECA) (K_i 1.1 nM) is 50-fold selective for rat brain A_3 receptors versus A_{2A} or A_1 receptors (Gallo-Rodriguez *et al.*, 1994) (fig. 2). The iodinated radioligand [^{125}I]AB-MECA binds with approximately nanomolar affinity to rat brain A_3 adenosine receptors expressed in CHO cells, but also binds to native A_1 receptors. Selectivity is increased by 2-substitution of N^6-benzyladenosine-5′-uronamides; 2-chloro-IB-MECA (2Cl-IB-MECA, K_i = 0.33 nM) is highly selective for A_3 versus A_1 and A_{2A} receptors, by 2500- and 1400-fold, respectively (Kim *et al.*, 1994) (fig. 2). There is pronounced interspecies differences in the relative affinities of agonist binding at A_3 receptors (Ji *et al.*, 1994; Linden, 1994).

F. Antagonists

Several classes of compounds have been developed as A_3 antagonists. One class comprises xanthines and their derivatives. Rat, rabbit, and gerbil brain A_3 receptors bind only weakly to xanthine derivatives compared with human and sheep A_3 receptors, which exhibit high affinity (Zhou *et al.*, 1992; Linden *et al.*, 1993; Salvatore *et al.*, 1993; Ji *et al.*, 1994). The most potent of the 8-phenyl-substituted xanthines, I-ABOPX (3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propylxanthine, or BW-A522) binds with nanomolar affinity to human and sheep A_3 receptors (Linden *et al.*, 1993; Salvatore *et al.*, 1993), but by contrast with micromolar affinity at rabbit, gerbil, and rat A_3 receptors (Ji *et al.*, 1994).

Five chemical classes of non-xanthine antagonists have been reported. L-268605 (3-(4-methoxyphenyl)-5-amino-7-oxo-thiazolo [3, 2]pyrimidine) is a potent and selective A_3 antagonist with a K_i value of 18 nM and no appreciable affinity for human A_1 and A_{2A} receptors (Jacobson *et al.*, 1996) (fig. 3). Another class is represented by L-249313 (6-carboxymethyl-5,9-dihydro-9-methyl-2-phenyl-[1, 2, 4]-triazolo[5,1-a][2, 7]naphthyridine) with high affinity at cloned human A_3 receptors, K_i value of 13 nM, but low affinity at native rat brain A_3 receptors, K_i 58 μM, and selectivity of approximately 300- and 1460-fold over A_1 and A_{2A} receptors, respectively (Jacobson *et al.*, 1996) (fig. 3).

The three other categories of molecules with promise as A_3 receptor antagonists are the flavonoid MRS 1067 (3,6-dichloro-2'isopropyloxy-4'-methyl-flavone), the 6-phenyl-1,4-dihydropyridines MRS 1097 (3,5-diethyl[2-methyl-6-phenyl-4-(2-phenyl-(*E*)-vinyl]-1,4-(±)-dihydropyridine-3,5-dicarboxylate) and MRS 1191 (3-ethyl 5-benzyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate) and the triazoloquinazolene MRS 1220 (9-chloro-2-(2-furyl)-5-phenylacetylamino[1, 2, 4]triazolo[1,5-c]quinazoline). Of these, MRS 1220 and MRS 1197 show promise as potent and selective competitive antagonists, with K_i values of 0.6 and 31 nM, respectively, for inhibition of [^{125}I]AB-MECA binding and K_B values of 1.7 and 92 nM at human recombinant A_3 receptors (Jacobson *et al.*, 1997). A much lower affinity was observed at the rat A_3 receptor: >2000-fold for MRS1220 and 112-fold for MRS 1197 (Jacobson *et al.*, 1997) as has been noted with xanthine-based antagonists.

G. Distribution and Biological Effects

The A_3 receptor is widely distributed, but its physiological role is still largely unknown. A_3 mRNA is expressed in testis, lung, kidneys, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, proximal colon, and eye of rat, sheep, and humans (Zhou *et al.*, 1992; Linden *et al.*, 1993; Salvatore *et al.*, 1993; Linden, 1994; Rivkees, 1994; Dixon *et al.*, 1996) (fig. 4). A_3 mRNA was not detected in rat skin or skeletal muscle (Dixon *et al.*, 1996) (fig. 4). Rat testis seems to have particularly high concentrations of A_3 mRNA (in spermatocytes and spermatids), compared with rather lower levels in most other rat tissues (Linden *et al.*, 1993; Salvatore *et al.*, 1993). The highest levels of human A_3 mRNA are found in lung and liver, with lower levels in aorta and brain (Salvatore *et al.*, 1993). In sheep, the highest levels of A_3 mRNA are found in lung, spleen, pars tuberalis, and pineal gland (Linden *et al.*, 1993). PCR was used to establish the presence of A_3 receptors in rabbit cardiac myocytes (Wang *et al.*, 1997).

The A_3 receptor on mast cells facilitates the release of allergic mediators including histamine, suggesting a role in inflammation (Ramkumar *et al.*, 1993b). Systemic administration of 3-IB-MECA causes scratching in mice that is prevented by coadministration of a histamine antagonist (Jacobson *et al.*, 1993b). APNEA has been shown to be a bronchoconstrictor in rats in vivo, an effect that may be mediated by mast cells (Pauwels and Joos, 1995), but it does not elicit bronchoconstriction in rabbits (el-Hashim *et al.*, 1996). Constriction mediated by adenosine in isolated arterioles of golden hamster cheek pouches is blocked by an inhibitor of mast cell degranulation, which suggests a role for A_3 receptors on mast cells in this response (Doyle *et al.*, 1994).

The A_3 receptor has been implicated in the 8-SPT-resistant hypotensive response to APNEA in the pithed rat (Fozard and Carruthers, 1993). The response is pertussis toxin-sensitive and is blocked by the A_3 receptor antagonist BW-A522 (Fozard and Hannon, 1994). However, it seems that the hypotensive response may be caused by the secondary action of histamine released after activation of mast cell A_3 receptors (Hannon *et al.*, 1995).

Systemic administration of 3-IB-MECA depresses locomotor activity in mice, which may suggest a role for brain A_3 adenosine receptors in modulation of behavior (Jacobson *et al.*, 1993b). Interestingly, activation of rat hippocampal A_3 receptors has been shown to desensitize A_1 receptor-mediated inhibition of excitatory neurotransmission in this brain region, indicating cross-talk between these two receptors (Dunwiddie *et al.*, 1997).

A_3 receptors on human eosinophils (Kohno *et al.*, 1996a) and human promyelocytic HL-60 cells (Kohno *et al.*, 1996b; Yao *et al.*, 1997) seem to be involved in apoptosis, an active self-destructive process caused by a genetically programmed cascade of molecular events involving DNA degradation and death of the cell by nuclear and cytoplasmic breakup. This seems to require high concentrations of agonist or chronic activation of the A_3 receptor in a manner that mimicks the requirement of high levels of ATP to activate the non-specific pore-formation of the $P2X_7$ receptor and apoptosis, and suggests that this potentially autocatalytic process may occur during pathological conditions resulting in cell damage and release of high levels of purines. Apoptotic effects are caused by high concentrations (micromolar) of A_3 receptor agonist in HL-60 leukemia and U-937

lymphoma cells, but paradoxically, A_3 receptor antagonists also induce apoptotic cell death, and this is opposed by low (nanomolar) concentrations of Cl-IB-MECA (Yao *et al.*, 1997). This indicates that low-level activation of A_3 receptors may result in cell protection, and furthermore that this may occur as a consequence of endogenously released adenosine (Yao *et al.*, 1997). Acute stimulation of A_3 receptors with micromolar concentrations of Cl-IB-MECA has also been shown to cause lysis of granular hippocampal neurons in culture (Von Lubitz *et al.*, 1996).

A_3 receptors may be involved in the cardioprotective effect of adenosine in ischemia and preconditioning during ischemia reperfusion injury (Liu *et al.*, 1994; Armstrong and Ganote, 1994, 1995; Auchampach *et al.*, 1997b; Stambaugh *et al.*, 1997). Preconditioning is blocked by A_3 receptor antagonists, whereas APNEA (A_1/A_3 selective), but not R-PIA (A_1 selective), protect against ischemia in rabbit cardiomyocytes (Armstrong and Ganote, 1995). A_3 receptors have been shown to mediate preconditioning and to reduce myocardial injury (Strickler *et al.*, 1996; Tracey *et al.*, 1997). In isolated cardiac myocytes, maximal preconditioning-induced cardioprotection was shown to require activation of both A_1 and A_3 receptors (Wang *et al.*, 1997). Acute IB-MECA has a detrimental effect on ischemic brain injury, whereas chronic IB-MECA has a protective effect (Von Lubitz *et al.*, 1994). This dual effect mimicks the effects of Cl-IB-MECA on leukemia and lymphoma cell lines (Yao *et al.*, 1997). Activation of an A_3 receptor in basophilic leukemia cells (RBL-2H3), endothelial cells, cardiac myocytes, and smooth muscle cells activates the cellular antioxidant defense system by increasing the activity of superoxide dismutase, catalase, and glutathione reductase, thereby providing a means by which adenosine may have a cytoprotective action in ischemia (Maggirwar *et al.*, 1994).

VII. Integrated Effects of Adenosine/P1 Receptors

A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors have distinct but frequently overlapping tissue distributions. The fact that more than one adenosine/P1 receptor subtype may be expressed by the same cell raises questions about the functional significance of this colocalization. Because the different adenosine/P1 receptor subtypes have quite different affinities for the endogenous agonist, the local concentration of adenosine in physiological and pathophysiological conditions is likely to be extremely important. EC_{50} values for adenosine at rat A_1, A_{2A}, A_{2B}, and A_3 receptors of 73 (Daly and Padgett, 1992), 150 (Daly and Padgett, 1992), 5100 (Peakman and Hill, 1994), and 6500 (Zhou *et al.*, 1992), respectively, have been reported. At rat phrenic motor nerve terminals (Correia-de-Sá *et al.*, 1996) and prejunctional receptors in rat vas deferens (Gonçalves and Queiroz, 1993), the concentration of adenosine needed to increase transmitter release via activation of A_{2A} receptors seems to be higher than that required to inhibit transmitter release via A_1 receptors. Because adenosine is formed as a breakdown product of ATP released from nerves, this implies that the adenosine concentration is crucially linked to the ongoing neuronal activity, which therefore may be an important determinant of the subtype of autoregulatory adenosine receptor that is activated. In rat hemidiaphragm, the frequency and intensity of stimulation of motor nerves and subsequent formation of endogenous adenosine was shown to be critical, with high-intensity, high-frequency nerve stimulation favoring A_{2A} receptor-mediated facilitation of [^{3}H]acetylcholine (ACh) release (Correia-de-Sá *et al.*, 1996). Thus, adenosine concentration and receptor affinity may determine the pattern of differential activation of coexpressed A_1 and A_{2A} receptors (and other adenosine receptors).

Expression of more than one type of adenosine/P1 receptor on the same cell may allow the common agonist adenosine to activate multiple signaling pathways. Adenylate cyclase is a common effector, which is negatively coupled to A_1 and A_3 receptors and positively coupled to A_2 receptors, affording the opportunity for reciprocal control and, therefore, fine tuning of this signaling pathway. Coexisting A_1 and A_2 adenosine receptors with opposite actions on adenylate cyclase activity have been described in a number of cells, including the smooth muscle cell line DDT_1 MF-2 (Ramkumar *et al.*, 1991), cultured porcine coronary artery smooth muscle cells (Mills and Gewirtz, 1990), and glomeruli and mesangial cells (Olivera and Lopez-Novoa, 1992). A_1 and A_{2B} receptors on primary rat astrocytes each regulate adenylate cyclase activity, but independently (Peakman and Hill, 1994).

The extracellular adenosine concentration may be a crucial determinant of the differential activation of coexisting adenosine/P1 receptors under pathophysiological as well as physiological conditions. Induction and inhibition of the inflammatory response by neutrophil A_1 and A_2 receptors, respectively, has been reported (Cronstein, 1994; Bullough *et al.*, 1995). Low concentrations of adenosine caused activation of the A_1 receptor and induced superoxide anion generation, phagocytosis via Fc receptors, and adhesion to endothelial cells, whereas higher concentrations of adenosine (>500 nM) required to saturate A_2 receptors lead to inhibition of these effects. A_{2A} and A_{2B} receptors coexist on fetal chick heart cells; the high affinity A_{2A} receptor has been suggested to be an important modulator of myocyte contractility under physiological conditions, whereas under pathophysiological conditions, such as cardiac ischemia resulting in release of large amounts of adenosine, the low affinity A_{2B} receptor may assume functional significance (Liang and Haltiwanger, 1995). Such studies are helping to expand on the established link between adenosine release and the metabolic demands of tissues by

building in specific actions on identified cell-surface adenosine/P1 receptors.

Stimulation of the A_{2A} receptor on rat striatal synaptosomes causes desensitization of coexpressed A_1 receptors, favoring A_{2A} receptor-mediated signaling (Dixon *et al.*, 1997a). This has important implications for other coexpressed adenosine receptors, and it would be interesting to see if this is a general phenomenon for these subtypes.

There is an interesting sidedness to the opposite responses evoked by A_1-like and A_{2A}-like adenosine receptors colocalized on monolayers of renal epithelial cells (Casavola *et al.*, 1997). The A_1-like receptors are located on the apical surface and mediate inhibition of transepithelial Na^+ transport by (*a*) inhibition of the basolaterally located Na^+/H^+ exchanger and (*b*) an increase in intracellular H^+, probably via Ca^{2+}/PKC. The A_{2A}-like receptors are located on the basolateral side and stimulate transepithelial Na^+ transport, suggested to be via stimulation of Na^+/H^+ exchange and thereby cellular alkalinization, probably via an increase in cAMP/PKA (Casavola *et al.*, 1997). The same adenosine receptor can elicit a different functional response in different tissues. In rat duodenum, A_{2B} (and A_1) adenosine receptors on the longitudinal muscle mediate relaxation, whereas A_{2B} receptors on the muscularis mucosae mediate contraction (Nicholls *et al.*, 1996).

Integrated effects of adenosine/P1 receptors in whole tissue responses are considered, together with P2 receptors, in Section XXII.

VIII. P2 Receptors

A. Introduction

P2 receptors are divided into two main classes based on whether they are ligand-gated ion channels (P2X receptors) or are coupled to G proteins (P2Y receptors) (Abbracchio and Burnstock, 1994; Fredholm *et al.*, 1994) (table 7).

The P2X/P2Y nomenclature was adopted from that originally used in a subdivision of P2 receptors proposed in 1985 by Burnstock and Kennedy, who described "P_{2X}-" and "P_{2Y}-purinoceptors" with distinct pharmacological profiles and tissue distributions: the "P_{2X}-purinoceptor" was shown to be most potently activated by the stable analogs of ATP, α,β-methylene ATP (α,β-meATP), and β,γ-meATP. At the "P_{2Y}-purinoceptor" 2-methylthio ATP (2MeSATP) was the most potent agonist and α,β-meATP and β,γ-meATP were weak or inactive. Furthermore, the "P_{2X}-purinoceptor" was shown to be selectively desensitized by α,β-meATP and to be antagonized by 3′-O-(3-[N-(4-azido-2-nitrophenyl)amino]-propionyl)ATP ($ANAPP_3$) (Burnstock and Kennedy, 1985). Distinct tissue distributions and functions reinforced this subdivision: "P_{2X}-purinoceptors" were shown to be present in vas deferens, urinary bladder, and vascular smooth muscle, and to mediate contraction; "P_{2Y}-purinoceptors" were shown to be present in guinea-pig taenia coli and on vascular endothelial cells, as well as to mediate relaxation. P2 receptors have since been cloned from smooth muscle and endothelium; the pharmacological profiles originally attributed to "P_{2X}-" and "P_{2Y}-purinoceptors" seem to correspond most closely to activation of $P2X_1$-like and $P2Y_1$–like receptors, respectively. However, it is now apparent that there is heterogeneity of P2X responses among different smooth muscles, and of P2Y responses between taenia coli and endothelium, which may be caused by different receptor subtypes or small differences in structure of the same receptor.

Other P2 receptors that have been identified in biological tissue principally according to their different pharmacological profiles are the P_{2U} receptor (activated equally by ATP and UTP; widely distributed), the P_{2T} receptor (platelet ADP receptor; mediates aggregation), and the P_{2Z} receptor (found on mast cells and lyphocytes; mediates cytotoxicity and degranulation) (Gordon, 1986; O'Connor *et al.*, 1991). P_{2S} (Wiklund and Gustafsson, 1988), P_{2R} (Von Kügelgen and Starke, 1990), P_{2D} (Pintor *et al.*, 1993), uridine nucleotide-specific receptors ("pyrimidinoceptors") (Seifert and Schultz, 1989; Von Kügelgen and Starke, 1990), P3 (Shinozuka *et al.*, 1988; Forsythe *et al.*, 1991), and P4 (Pintor and Miras-Portugal, 1995a) receptors have also been proposed. Of these the P_{2U}, P_{2Z}, and uridine nucleotide-specific receptors have been cloned. Because receptor subclassification based on pharmacological criteria alone is no longer tenable, the separate identity of the other proposed subtypes remains to be proved.

The revision of P2 receptor nomenclature was prompted by evidence that extracellular ATP works through two different transduction mechanisms, namely intrinsic ion channels and G protein-coupled receptors (Benhan and Tsien, 1987; Dubyak, 1991), and by the cloning of the first two P2 receptors, $P2Y_1$ (a "P_{2Y}-purinoceptor") (Webb *et al.*, 1993b) and $P2Y_2$ (a "P_{2U}-purinoceptor") (Lustig *et al.*, 1993). It was also becoming increasingly apparent that there was significant heterogeneity among native P2 receptors, reflected in an increasing diversity of pharmacological response profiles that could not easily be accommodated within the existing system of receptor subclassification. Thus, in 1994 it was formally suggested that P2 receptors should be divided into two broad groups termed P2X and P2Y according to whether they are ligand-gated ion channels or are coupled to G proteins, respectively, with subtypes defined by the different structure of mammalian P2 receptors (Abbracchio and Burnstock, 1994; Barnard *et al.*, 1994; Fredholm *et al.*, 1994).

To date seven mammalian P2X receptors, $P2X_{1-7}$, and five P2Y receptors, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ have been cloned, characterized pharmacologically and accepted as valid members of the P2 receptor family. The use of lower case to define the cloned p2y3 receptor reflects the possibility that this may be the avian ho-

TABLE 7
P2 receptor signal transduction mechanisms, agonists, and antagonists

Family		P2X	P2Y
Receptor type		Ion channel Nonselective pore[a]	G protein-coupled: $G_{q/11}$, G_i[b]
Signaling pathway		N.A.	PLC, AC,[c] K^+ channels[d] PLC_{PC},[e] PLA_2,[f] PLD[f] PKC MAPK[g]
Effectors		$Ca^{2+} \gg Na^+ > K^+$	↑ IP_3, ↑ Ca^{2+}, ↑ DAG ↓ cAMP[c] Ca^{2+}, Cl^-, K^+ currents[h]
Agonists	*Nonselective*	ATP ATPγS 2MeSATP Ap_4A	ATP[i] ATPγS[j] 2MeSATP[k] Ap_4A[j]
	P2X/P2Y-selective	α,β-meATP[l] β,γ-meATP[l] BzATP[a]	ADP[e] UTP[m] UTPγS[j] UDP[n] 2Cl-ADP[e] 2MeSADP[e] ADPβS,[e] ADPβF[e]
Antagonists	*Nonselective*	Suramin PPADS Reactive blue 2	Suramin PPADS Reactive blue 2
	P2X/P2Y-selective	NF023 NF279 KN-62[a]	ARL 67085[o] FPL 66096[o] A3P5PS[k] MRS 2179[k] 2-hexylthio-ATP[p] 2-cyclohexylthio-ATP[p]

N.A., not applicable.
[a] $P2X_7$ and endogenous $P2X_7$-like receptor.
[b] $P2Y_1$ and endogenous $P2Y_1$-like receptors acting through PLC couple to $G_{q/11}$ proteins; $P2Y_1$ and endogenous $P2Y_1$-like receptors acting through adenylate cyclase couple to G_i proteins; $P2Y_2$ and endogenous $P2Y_2$-like receptors, $P2Y_4$ and $P2Y_{ADP}$ receptors couple to $G_{q/11}$ and G_i proteins; p2y3 and $P2Y_6$ receptors couple to $G_{q/11}$ proteins.
[c] $P2Y_1$ and endogenous $P2Y_1$-like receptors and $P2Y_{ADP}$ receptors.
[d] Some endogenous $P2Y_1$-like receptors activate K^+ channels via interactions with their G protein subunits.
[e] $P2Y_1$ and endogenous $P2Y_1$-like receptor signaling; possibly downstream of PKC.
[f] $P2Y_1$ and $P2Y_2$ receptors and their endogenous counterparts; signaling possibly downstream of PKC.
[g] $P2Y_1$ and $P2Y_2$ receptors and their endogenous counterparts; signaling downstream of PKC.
[h] Secondary to activation of PLC, although activation of K^+ currents by some endogenous $P2Y_1$-like receptors is via direct interactions with G protein subunits.
[i] $P2Y_1$ and $P2Y_2$ receptors and their endogenous counterparts; ATP is an antagonist at $P2Y_{ADP}$ receptors.
[j] $P2Y_2$ and endogenous $P2Y_2$-like receptors.
[k] $P2Y_1$ and endogenous $P2Y_1$-like receptors.
[l] $P2X_1$, $P2X_3$ and heteromeric $P2X_2P2X_3$ receptors.
[m] $P2Y_2$ and endogenous $P2Y_2$-like receptors and $P2Y_4$ receptors.
[n] $P2Y_6$ receptor.
[o] $P2Y_{ADP}$.
[p] $P2Y_1$ and endogenous $P2Y_1$-like receptors coupled to AC.
Abbreviations: AC, adenylate cyclase; ADPβF, adenosine 5′-*O*-(2-fluoro)-diphosphate; ADPβS, adenosine 5′-*O*-(2-thio-diphosphate; cAMP, adenosine 3′,5′-cyclic monophosphate; A3P5PS, adenosine 3′-phosphate 5′-phosphosulfate; ARL 67085, 6-*N*,*N*-diethyl-D-β,γ-dibromomethylene ATP; ATPγS, adenosine 5′-*O*-(3-thiotriphosphate); BzATP, 3′-*O*-(4-benzoyl)benzoyl ATP; DAG, diacylglycerol; FPL 66096, 2-propylthio-D-β,γ-difluoromethylene ATP; IP_3, inositol 1,4,5-trisphosphate; KN-62, 1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine; MAPK, mitogen-activated protein kinase; α,β-meATP, α,β-methylene ATP; β,γ-meATP, β,γ-methylene ATP; 2MeSADP, 2-methylthio ADP; 2MeSATP, 2-methylthio ATP; MRS 2179, N^6-methyl modification of 2′-deoxyadenosine 3′,5′-*bis*phosphate; NF023, symmetrical 3′-urea of 8-(benzamido)naphthalene-1,3,5-trisulfonic acid; NF279, 8,8′-(carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid); PLC_{PC}, phosphatidylcholine-specific phospholipase C; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PPADS, pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid; suramin, 8-(3-benzamido-4-methylbenzamido)-naphthalene-1,3,5-trisulfonic acid; UTPγS, uridine 5′-*O*-(3-thiotriphosphate).

molog of the human $P2Y_6$ receptor, although this has not yet been confirmed. The jump in sequence in the numbering of the P2Y receptor family is caused by the recognition that certain receptors had been erroneously identified as belonging to this family, leading to the subsequent withdrawal of $P2Y_5$ (Webb *et al.*, 1996b) and $P2Y_7$ (Akbar *et al.*, 1996). The cloned receptors $P2Y_9$ and $P2Y_{10}$ are also not nucleotide receptors. A P2Y receptor cloned from *Xenopus* neural plate (provisionally called $P2Y_8$) is not included in the definitive P2Y receptor family recognized by the IUPHAR committee, based largely on the rationale that this is a non-mammalian receptor. The platelet ADP receptor $P2Y_{ADP}$ (or P_{2T} receptor) has not yet been cloned and, therefore, as recommended by the IUPHAR committee, the name of this receptor is given in italics.

$P2Y_4$ (human but not rat receptor) and $P2Y_6$ are uridine nucleotide-specific receptors (receptors not activated or only weakly activated by purines) that have been cloned and shown to be sensitive preferentially to UTP and UDP, respectively (the rat $P2Y_4$ receptor is also activated potently by ATP; see Section XV). Their identification complements earlier suggestions of the existence of endogenous uridine nucleotide-specific receptors based on distinct pharmacology of some biological tissue. Before the cloning of these receptors, the possibility that there were subtypes of endogenous uridine nucleotide-specific receptors was not considered,

and by implication the possibility of different UTP/UDP selectivities for members of this family was not appreciated. Thus, in much of the literature to date, the agonist potency profiles documented for endogenous uridine nucleotide-specific receptors are incomplete, leaving open the possibility that these are $P2Y_4$ or $P2Y_6$ receptors, or some other subtype not yet cloned. The lack of selective agonists and antagonists, and complications introduced by receptor coexpression and agonist interconversion, means that the subtype identity of most endogenous uridine nucleotide-specific receptors is currently unclear. Because of this, a separate section in this review is devoted to endogenous uridine nucleotide-specific receptors (see Section XVIII.). Interestingly, the $P2Y_{11}$ receptor is so far the only P2Y receptor selective for ATP versus other purine and pyrimidine nucleotides.

For researchers in this field, important discoveries made in the last 10 years have been the source of insight, and in some cases frustration, because these demand a reevaluation of conclusions drawn from earlier studies on P2 receptors. These include the discovery that: (*a*) multiple P2X receptor proteins are often coexpressed in different proportions in different tissues; (*b*) P2X receptors are multisubunit receptors that may exist as heteromers with different pharmacology compared with the homomers; (*c*) cations can profoundly affect P2X channel activity; (*d*) 2MeSATP, previously widely regarded as a selective "P_{2Y}-purinoceptor" agonist, is also a potent agonist at P2X receptors; (*e*) ecto-nucleotidases can profoundly alter agonist potencies; and (*f*) antagonists previously used with some confidence as P2 receptor blockers are non-selective, can modulate ecto-nucleotidase activity and may have allosteric effects on P2 receptors. The general lack of selective agonists and antagonists, together with complications introduced by coexistence of different P2 receptors and impure solutions caused by purine and pyrimidine degradation and interconversion, also has significantly hindered advances in P2 receptor characterization.

Although much valuable information can be derived from studies of populations of cells in culture, there are potential pitfalls associated with this technique. Thus, emerging evidence that the expression of P2 receptors may alter in culture conditions adds another potential complication to the study of purine receptors. For example, astrocytes studied in situ, or after acute isolation from rat brain, are insensitive or only a few cells respond to ATP, whereas in primary cultures, there is a profound increase in the number of cells responding to ATP (Jabs *et al.*, 1997; Kimelberg *et al.*, 1997). Similarly, up-regulation of the $P2Y_2$ receptor in rat salivary gland cells in culture compared with acutely isolated cells has been reported (Turner *et al.*, 1997). Thus, caution is needed in the interpretation of studies of P2 receptors on cells in culture.

Autocatalytic release of ATP has been shown from endothelial cells (Yang *et al.*, 1994) and it is possible that this phenomenon will be described for other cell types as well as for other purines and pyrimidines. In addition, ATP is released from many different cells in response to stimuli such as shear stress and hypoxia, which may be relevant for the ongoing level of activation of purine receptors expressed by the same or neighboring cells. This may be particularly important with respect to the activity of $P2X_1$ and $P2X_3$ receptors, as these receptors desensitize rapidly.

Because of the diverse reasons discussed above, it is currently a considerable challenge to dissect out and characterize endogenous receptors for purines and pyrimidines in different biological systems, and even more of a challenge to identify for each of these a physiological or pathophysiological role. However, endogenous receptor counterparts have been shown for some cloned P2 receptors, matching both in terms of receptor distribution, signaling mechanisms, and pharmacology. In this review, we use the name of the clone in preference to the classical nomenclature where possible to promote the conversion from the older system to the newer terminology. However, because for the majority of cases this characterization is currently equivocal, we qualify this with the term "-like". Thus, "$P2X_1$-like receptor" replaces the classical "P_{2X}-purinoceptor" of smooth muscle, "$P2X_7$-like receptor" is used for the "P_{2Z}-purinoceptor", "$P2Y_1$-like receptor" is used in preference to the classical "P_{2Y}-purinoceptor," and "$P2Y_2$-like receptor" replaces "P_{2U}-purinoceptor". Unequivocal characterization of endogenous P2 receptors awaits the development and use of subtype-selective agonists and antagonists.

B. Agonists

P2 receptors have broad natural ligand specificity, recognizing ATP, ADP, UTP, UDP, and the diadenosine polyphosphates (table 7). The chemical structures of some principal P2 receptor agonists are illustrated in figure 6. At present there are no agonists or antagonists that discriminate adequately between families of P2X and P2Y receptors, or between subtypes of receptors within each of these groups (table 7). Some of the most useful agonists are the stable ATP analogs α,β-meATP and β,γ-meATP, which if effective, strongly imply actions at P2X receptors (specifically at $P2X_1$ and $P2X_3$ subtypes) and are generally inactive at P2Y receptors. Also useful are ADP, adenosine 5′-O-(2-thiodiphosphate)(ADPβS,) and UTP, as these are agonists at some P2Y receptors, but are weak or inactive at P2X receptors.

Agonist potency orders, important in the characterization of cloned and native P2 receptors, are profoundly influenced by the different stabilities of P2 receptor ligands in the presence of ecto-nucleotidases. α,β-MeATP is considerably more stable than ATP and 2MeSATP when ecto-nucleotidase activity is not suppressed, which contributes significantly to its greater potency (up to 100-fold more potent) at native $P2X_1$ receptors in vascular smooth muscle, bladder, and vas deferens. However,

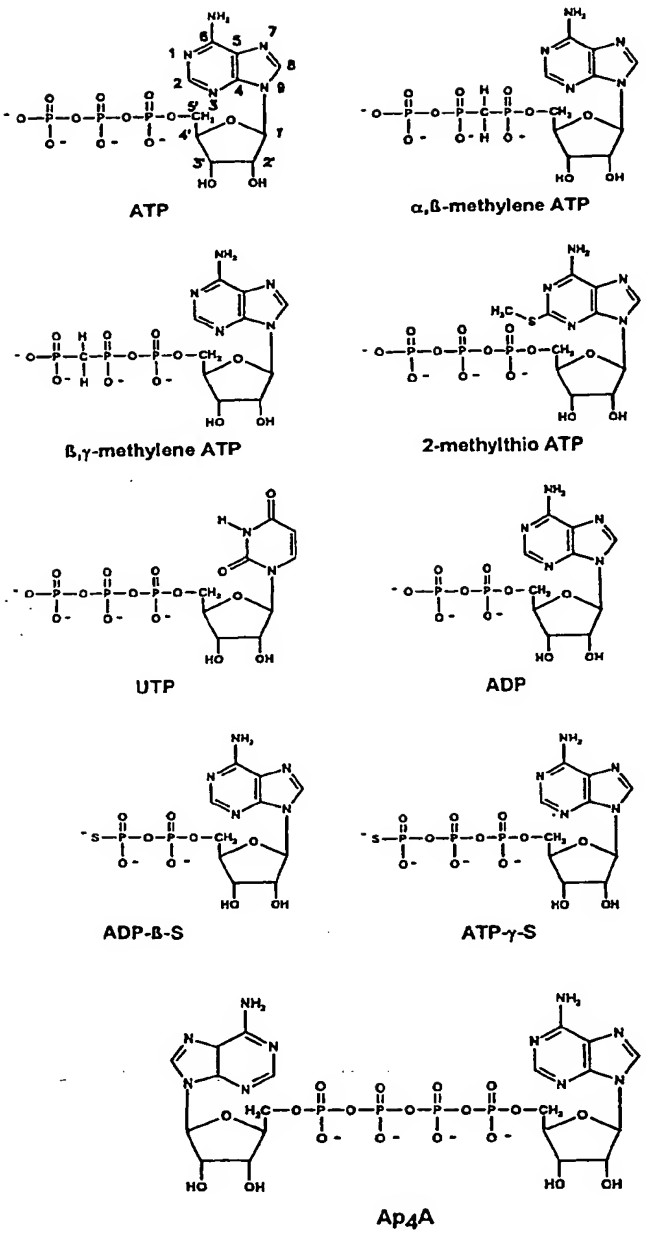


FIG. 6. The chemical structure of some key agonists at P2 receptors. (Adapted from Windscheif, 1996).

when ecto-ATPase effects are controlled by use of single cells and rapid concentration-clamp applications of agonist, or by inhibition of ecto-ATPase activity [for instance using 6-N,N-diethyl-D-β,γ-dibromomethylene ATP (ARL 67156) or removal of divalent cations], α,β-meATP is less potent than 2MeSATP and ATP at native and cloned $P2X_1$ receptors (Crack *et al.*, 1994; Evans and Kennedy, 1994; Humphrey *et al.*, 1995; Khakh *et al.*, 1995b). Thus, greater caution is now advised in the interpretation of the order of agonist potency where ecto-nucleotidase activity has not been suppressed. This is a particularly important consideration in the pharmacology of P2X receptors because of the wide range of stabilities of commonly-used P2X agonists, but seems to have had less of an impact on P2Y receptor profiles, probably because many of the commonly used P2Y agonists are similarly unstable. An additional consideration is that many P2 receptor antagonists inhibit ecto-nucleotidase activity, which may reduce their effectiveness against biologically unstable P2 agonists.

C. Antagonists

Antagonists selective for subtypes of P2X and P2Y receptors are considered in later sections on individual receptors (see Sections X.F., XII.E., XVIV.D.). This section considers other established and putative P2 receptor antagonists, which, unfortunately, do not discriminate well, if at all, between P2X or P2Y receptors, let alone for subtypes within these families (table 7). Many of these also inhibit ecto-nucleotidases and may have allosteric effects on the receptor (Michel *et al.*, 1997). Table 8 summarizes the potencies of some of the most commonly used antagonists at recombinant and endogenous P2 receptors. The general lack of selective antagonists highlights the problems currently encountered in subtype-identification of P2 receptors using ligand binding. The chemical structures of some P2 receptor antagonists are illustrated in figure 7.

In principle, any P2 receptor antagonist should be tested for its selectivity against all known subtypes of this family. Evaluation of antagonist selectivity at heteromeric P2X receptors is also important because of its relevance for biological tissue where P2X receptor proteins are typically coexpressed; such studies might additionally provide useful information about the specific contribution of the different subunits to the pharmacology of the receptor heteromer. A commonly used biological assay is antagonism of constriction by α,β-meATP of vas deferens and vascular smooth muscle. This is generally taken as an indication of actions at endogenous $P2X_1$-like receptors for a number of reasons: (*a*) the $P2X_1$ receptor has been cloned from smooth muscle; (*b*) immunohistochemical studies have shown that it is the predominant P2X receptor protein expressed by smooth muscle; (*c*) α,β-meATP is selective for $P2X_1$ and $P2X_3$ receptors, but the latter is not expressed by smooth muscle; and (*d*) the smooth muscle P2X response shows a similar pharmacology to the recombinant $P2X_1$ receptor, and as with the $P2X_1$ receptor, undergoes rapid desensitization. Relaxant effects of 2MeSATP or ADPβS at guinea-pig taenia coli and via the vascular endothelium have been used to examine antagonist potencies at endogenous $P2Y_1$-like receptors. The potencies of antagonists at endogenous P2 receptors in these and other biological assays are reported in table 8b.

1. Suramin. The trypanoside suramin (8-(3-benzamido-4-methylbenzamido)-naphthalene-1,3,5-trisulfonic acid) is generally selective as an antagonist at P2

TABLE 8a
Antagonist selectivities at cloned P2 receptors

Receptor	Suramin	PPADS	P5P	RB2	NF023	References[g]
$P2X_1$	IC_{50} 1–5	IC_{50} 1	IC_{50} 10–20	N.D.	N.D.	1,2
$P2X_2$	IC_{50} 1–5	IC_{50} 2	IC_{50} 10–20	Yes	N.D.	1,2
$P2X_3$	IC_{50} 3	IC_{50} 1	IC_{50} 10	N.D.	N.D.	1
$P2X_{4\ rat}$	Inactive (>500 μM)	Inactive (>100 μM)	Inactive (>100 μM)	Inactive[a] (>50 μM) IC_{50} 46–50[b] IC_{50} 120–128[c]	N.D.	1,3–6
$P2X_{4\ human}$	IC_{50} 178	IC_{50} 27.5		IC_{50} 38[b] IC_{50} 39[c]		
$P2X_5$	IC_{50} 4	IC_{50} 3	N.D.	N.D.	N.D.	1
$P2X_6$	Inactive	Inactive	N.D.	N.D.	N.D.	1
$P2X_{7\text{-}human}$	IC_{50} 4	IC_{50} 4.2	N.D.	N.D.	N.D.	7,8
$P2X_{7\text{-}rat}$	IC_{50} 4.1	IC_{50} 4.3				
$P2Y_1$	pA_2 5.4–6	pA_2 6	N.D.	N.D.	N.D.	9–11
$P2Y_2$	pA_2 4.3	Inactive	N.D.	N.D.	N.D.	10
p2y3	pA_2 5	N.D.	N.D.	N.D.	N.D.	12
$P2Y_{4\text{-}human}$	Inactive	IC_{50} 15/inactive	N.D.	Yes[d]	N.D.	14,15
$P2Y_{4\text{-}rat}$	Weak	Inactive	N.D.	IC_{50} 21	N.D.	16
$P2Y_6$	Slight[e]	Slight[f]	N.D.	IC_{50} 31	N.D.	15,17
$P2Y_{11}$	N.D.	N.D.	N.D.	N.D.	N.D.	18

IC_{50} and pA_2 values are μM; N.D., not determined.
[a] RB2.
[b] Basilen blue (isomer of RB2).
[c] Cibacron blue (isomer of RB2).
[d] 33% inhibition of the UTP response.
[e] Less potent than RB2 and PPADS.
[f] Less potent than RB2.
[g] References: 1 Collo *et al.*, 1996; 2 Evans *et al.*, 1995; 3 Bo *et al.*, 1995; 4 Soto *et al.*, 1996a; 5 Buell *et al.*, 1996b; 6 Garcia-Guzman *et al.*, 1997a; 7 Surprenant *et al.*, 1996; 8 Rassendren *et al.*, 1997; 9 Brown *et al.*, 1995; 10 Charlton *et al.*, 1996a; 11 Schachter *et al.*, 1996; 12 Webb *et al.*, 1996a; 13 Charlton *et al.*, 1996b; 14 Communi *et al.*, 1996a; 15 Chang *et al.*, 1995; 16 Bogdanov *et al.*, 1998; 17 Robaye *et al.*, 1997; 18 Communi *et al.*, 1997.

receptors versus other types of receptors (Dunn and Blakeley, 1988) (but see later this section), but is not a universal P2 receptor antagonist, and does not discriminate between P2X and P2Y receptors (table 8). Furthermore, suramin inhibits ecto-nucleotidase (Crack *et al.*, 1994; Beukers *et al.*, 1995; Ziganshin *et al.*, 1995; Bültmann *et al.*, 1996b; Chen *et al.*, 1996c) and neural ecto-diadenosine polyphosphate hydrolase (Mateo *et al.*, 1996) activity, which may complicate interpretation of antagonist activity when it is used against ligands which are biologically unstable.

Antagonism by suramin of recombinant and endogenous P2X and P2Y receptors occurs with relatively low potency (pA_2 values approximately 5) (table 8). Antagonism is frequently non-competitive. Suramin is weak or inactive at recombinant $P2X_6$ and $P2X_4$ receptors (Buell *et al.*, 1996b) and at $P2Y_6$ and human $P2Y_4$ receptors (Chang *et al.*, 1995; Communi *et al.*, 1996a; Robaye *et al.*, 1997). Suramin is an antagonist at a subpopulation of endogenous $P2Y_2$-like receptors (Hoiting *et al.*, 1990; Murrin and Boarder, 1992; Henning *et al.*, 1992, 1993; Carew *et al.*, 1994; Chen *et al.*, 1994b; Sipma *et al.*, 1994; Ho *et al.*, 1995; Paulais *et al.*, 1995; Ziyal, 1997), and blocks native $P2X_7$ (or P_{2Z}) receptors in human lymphocytes (Wiley *et al.*, 1993).

Inhibition by suramin of nicotinic receptors in chick cultured sympathetic neurons (Allgaier *et al.*, 1995b), GABA and glutamate receptors in rat hippocampal neurons (Nakazawa *et al.*, 1995), and vasoactive intestinal polypeptide (VIP)- and 5-hydroxytryptamine (5-HT)-mediated relaxations of the guinea-pig proximal colon (Briejer *et al.*, 1995) have been described, at concentrations within the range used for block of P2 receptors. Suramin at 100 μM inhibits, by approximately 40%, GABA and glutamate receptor currents in rat hippocampal neurons (Nakazawa *et al.*, 1995), and 300 μM suramin produces approximately 40% block of 1,1-dimethyl-4-phenylpiperazinium (DMPP; nicotinic receptor agonist)-induced overflow of [^{3}H]NA in cultured chick sympathetic neurons (Allagaier *et al.*, 1995b). Inhibition by suramin of NMDA-gated ion channels (IC_{50} 68 μM) was described in mouse hippocampal neurons (Peoples and Li, 1998). In guinea-pig proximal colon, 300 μM suramin is a more potent inhibitor of relaxant responses to VIP (virtually abolishing responses) than of responses to ATP, and also produces a modest block of 5-HT-induced relaxation (Briejer *et al.*, 1995).

Other diverse effects of suramin include inhibition of the binding of growth factors, inhibition of the GTPase activity of certain G proteins, and inhibition of DNA and RNA polymerases (see Voogd *et al.*, 1993). Suramin and its analogs have been shown to block responses at A_1 adenosine and D_2 dopamine receptors in brain membranes by inhibiting the formation of the agonist/receptor/G protein complex (Beindl *et al.*, 1996). Although this should be borne in mind when interpreting the effects of suramin in biological systems, it should be noted that these studies were carried out on brain membrane preparations and that because of its highly polar nature, suramin does not readily cross cell membranes.

2. NF023. NF023 (symmetrical 3′-urea of 8-(benzamido)naphthalene-1,3,5-trisulfonic acid) is a suramin-

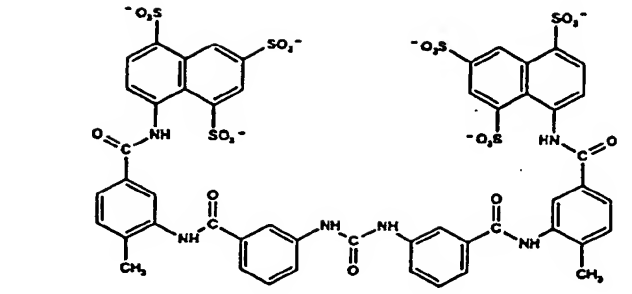
TABLE 8b
Antagonist selectivities at endogenous P2 receptors

Tissue	Receptor[a]	Suramin	PPADS	P5P	RB2	NF023	References[k]
Rat vas deferens	P2X ($P2X_1$)	pK_B 5.5 K_d 3.9	iso-PPADS[b]	pK_B 5.3–5.8	pK_B 5.8[c]	pA_2 5.9 K_d 1.0	1,2 3
Rabbit vas deferens	P2X ($P2X_1$)	pA_2 5.1	pA_2 6.3	pA_2 5.2		pA_2 5.7	4,5
Guinea-pig vas deferens	P2X ($P2X_1$)	Yes (NC)	pK_B 5.6				6,7
Rat mesenteric bed	P2X ($P2X_1$)	pA_2 5.0	pK_B 6.4	pA_2 5.4		pA_2 5.5	5,8
Hamster mesenteric bed	P2X ($P2X_1$)	pA_2 5.3				pA_2 5.6	9
Rabbit ear artery	P2X ($P2X_1$)	pK_B 4.8	pA_2 6.4			N.D.	10–12
Rabbit saphenous artery	P2X ($P2X_1$)	pA_2 4.8	pA_2 6.0			pA_2 5.7	9,11,12
Rat renal vascular bed	P2X ($P2X_1$)	Yes	pK_B 6.0		Yes		13
Guinea-pig ileum submucosal arterioles	P2X ($P2X_1$)	pK_B 5.5	pK_B 6.3				14
Rabbit urinary bladder	P2X ($P2X_1$)		pA_2 6.3				15
Guinea-pig urinary bladder	P2X ($P2X_1$)	pA_2 5.1	pA_2 6.7				16
Human urinary bladder	P2X ($P2X_1$)	pK_B 5.9[d]					17
Human saphenous vein	P2X ($P2X_1$)	pK_B 4.8					18
Rat vagus nerve	P2X	pA_2 5.9	iso-PPADS[e]	pK_B 5.3–5.4	pK_B 4.96[e]		19,20
Guinea-pig taenia coli	P2Y ($P2Y_1$)	pA_2 5.0 K_d 10.1	pA_2 4.6–5.3			pA_2 4.2 K_d 22–34	9,21,22 3
Rat duodenum	P2Y ($P2Y_1$)	pA_2 5.0	pA_2 5.1	pA_2 5.4		pA_2 4.3	5,11
Rat mesenteric bed	P2Y ($P2Y_1$)	pA_2 5.3	pA_2 5.5–6.0			pA_2 4.9	9,11,22,23
Bovine aorta	P2Y ($P2Y_1$)	pK_B 5.5					24
Rat aorta	P2Y ($P2Y_1$)	K_d 2–6	K_d 0.2–0.4		K_d 0.5–0.8		25
Turkey erythrocytes	P2Y ($P2Y_1$)	Yes (NC)	pA_2 5.9		Yes (NC)		26
Bovine pulmonary artery EC	P2Y ($P2Y_1$)	pA_2 5.5			pA_2 6.3		27
Rabbit thoracic aorta							9
$+EC_{(ATP)}$	P2Y ($P2Y_1$)	pA_2 3.2–4.4				Inactive	
$-EC_{(ATP)}$	P2Y	Inactive				Inactive	
C6 glioma cells							26,28
↑ IP_3	P2Y ($P2Y_1$)	pA_2 4.4			Yes (NC)		
↓ cAMP	P2Y ($P2Y_1$)	Slight at 100 μM	Inactive at <100 μM		pA_2 6.3		
Rat astrocytes	P2Y	Yes	IC_{50} 0.9				29
Mouse vas deferens	P2Y-like[f]				pK_B 5.3		30
Rat atria	P2Y-like[g]				pK_B 5.1		31
Rat mesenteric bed	P2Y ($P2Y_2$)	Inactive	Inactive			Inactive	9,23
Hamster mesenteric bed	P2Y ($P2Y_2$)	pA_2 4.9				Inactive	9
Bovine aorta	P2Y ($P2Y_2$)	Inactive					24
Rat aorta	P2Y ($P2Y_2$)	K_d 26–37			K_d 6.5		25
Bovine pulmonary artery EC	P2Y ($P2Y_2$)	pA_2 4.4			pA_2 5.7		27
C6 glioma	P2Y ($P2Y_2$)	pA_2 4.4					26
C2C12 myotubes	P2Y ($P2Y_2$)	pA_2 4.4					32
Rat astrocytes	P2Y ($P2Y_2$)	Yes	IC_{50} 7.2				29
Rat neuroblastoma × glioma cells	P2Y ($P2Y_2$)	IC_{50} 40–60	IC_{50} 20–30				33
RAW 264.7 macrophages	P2Y (pyrimidinoceptor)	pA_2 4.8	Inactive		pA_2 5.8		34
Rat mesenteric arteries	P2Y (pyrimidinoceptor)[h]	Inactive	Inactive		Inactive		22,23,35
Rat superior cervical ganglion	P2Y (pyrimidinoceptor)[i]	Inactive					36
Human platelets	P2Y (P_{2T})	pA_2 4.6	Yes[j]				37,38

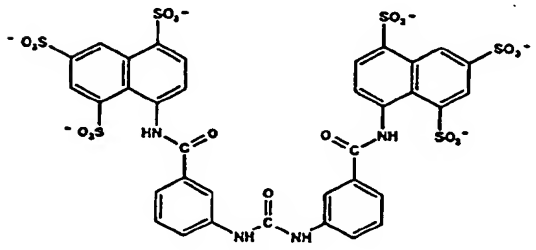
$P2X_1$-like receptor-mediated responses were determined against the effects of α,β-meATP; $P2Y_1$-like receptor-mediated responses were determined against the effects of ADPβS or 2MeSATP; $P2Y_2$-like receptor-mediated responses were determined against the effects of UTP (in tissues in which ATP is approximately equipotent with UTP). NC, noncompetitive; +EC, with endothelium; -EC, without endothelium.

[a] The likely cloned receptor counterparts of endogenous responses are indicated in parentheses.
[b] pK_B 6.6 for iso-PPADS (Khakh *et al.*, 1994).
[c] Cibacron blue.
[d] Suramin antagonized only the lower part of the α,β-meATP response curve (Palea *et al.*, 1995).
[e] pK_B 6.0 for iso-PPADS (Trezise *et al.*, 1994c).
[f] Antagonism of ATPγS inhibition of [^{3}H]NA overflow.
[g] Antagonism of ATP- and ATPγS-mediated inhibition of evoked [^{3}H]NA overflow.
[h] Tested against contractions to UTP.
[i] Tested against depolarizations to UDP and UTP; responses to α,β-meATP and ATP were blocked.
[j] At high concentrations (>100 μM).
[k] References: 1, Khakh *et al.*, 1994; 2, Trezise *et al.*, 1994b; 3, Bültmann *et al.*, 1996b; 4, Lambrecht *et al.*, 1992; 5, Lambrecht *et al.*, 1996; 6, McLaren *et al.*, 1994; 7, Bailey and Hourani, 1995; 8, Windscheif *et al.*, 1994; 9, Ziyal, 1997; 10, Leff *et al.*, 1990; 11, Lambrecht, 1996; 12, Ziganshin *et al.*, 1994b; 13, Eltze and Ullrich, 1996; 14, Galligan *et al.*, 1995; 15, Ziganshin *et al.*, 1993; 16, Usune *et al.*, 1996; 17, Palea *et al.*, 1995; 18, von Kügelgen *et al.*, 1995; 19, Trezise *et al.*, 1994b; 20, Trezise *et al.*, 1994c; 21, Hoyle *et al.*, 1990; 22, Windscheif *et al.*, 1995a; 23, Ralevic and Burnstock, 1996a; 24, Wilkinson *et al.*, 1994; 25, Hansmann *et al.*, 1997; 26, Boyer *et al.*, 1994; 27, Chen *et al.*, 1996a; 28, Lin and Chuang, 1994; 29, Ho *et al.*, 1995; 30, von Kügelgen *et al.*, 1994; 31, von Kügelgen *et al.*, 1995b; 32, Henning *et al.*, 1993; 33, Reiser, 1995; 34, Chen *et al.*, 1996c; 35, Lagaud *et al.*, 1996; 36, Connolly and Harrison, 1995; 37, Hourani *et al.*, 1992; 38, Windscheif *et al.*, 1995b.

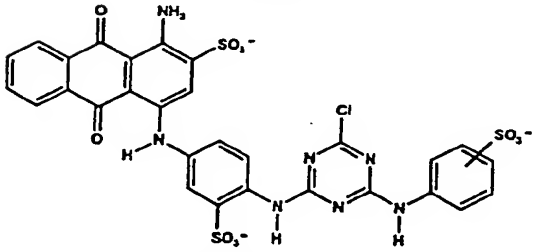
based compound which is moderately selective as an antagonist of P2X receptors. NF023 is about 30-fold selective for $P2X_1$-like receptors in the rat vas deferens versus $P2Y_1$-like receptors in the guinea-pig taenia coli (Bültmann *et al.*, 1996b). It has 79-fold selectivity for endogenous $P2X_1$-like receptors in rabbit vas deferens versus $P2Y_1$-like receptors in turkey erythrocytes; pA_2 values of 5.5 to 5.7 at $P2X_1$-like receptors in rabbit



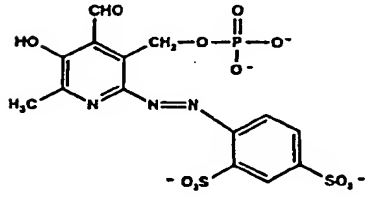
suramin



NF023



reactive blue 2



PPADS

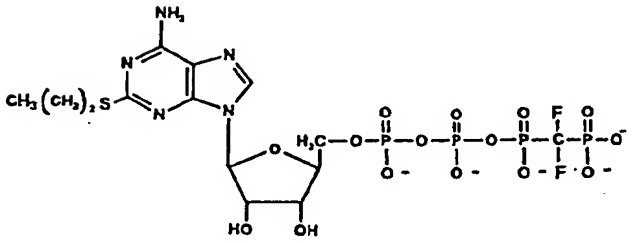


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FIG. 7. The chemical structures of some P2 receptor antagonists. (Adapted from Windscheif, 1996).

isolated blood vessels, rabbit vas deferens, and rat and hamster mesenteric arterial beds; and pA_2 values of 4.6 to 5.5 at vascular and nonvascular smooth muscle $P2Y_1$-like receptors (Lambrecht *et al.*, 1996; Ziyal, 1997; Ziyal *et al.*, 1997). Its effects at the other P2X (and P2Y) receptor subtypes have not been reported. Antagonism is competitive and reversible. Like the parent compound suramin, NF023 inhibits ecto-nucleotidase activity, but unlike suramin, it has high $P2X_1$-like versus ecto-nucleotidase-selectivity (Beukers *et al.*, 1995; Bültmann *et al.*, 1996b).

3. NF279. NF279 (8, 8′-(carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid) is a suramin analog that is about 10-fold more potent than NF023 in blocking α,β-meATP-mediated contractions at $P2X_1$-like receptors in rat vas deferens, pIC_{50} 5.7 (Damer *et al.*, 1998). With a pA_2 value of 4.3 at $P2Y_1$-like receptors in the guinea-pig taenia coli, it has the highest P2X- versus P2Y- and ecto-nucleotidase-selectivity so far reported (Damer *et al.*, 1998).

4. Pyridoxal-5-phosphate (P5P). P5P is a non-selective P2 receptor antagonist, but has proved useful as a starting compound for the synthesis of more P2X-selective antagonists (Lambrecht *et al.*, 1996). Antagonism by P5P is, however, selective versus non-purine receptors and seems to be competitive at $P2X_1$-like receptors in vas deferens of rabbit (Lambrecht *et al.*, 1996) and rat (Trezise *et al.*, 1994b), and at α,β-meATP-mediated depolarization of rat vagus nerve (Trezise *et al.*, 1994b). P5P non-competitively inhibits responses mediated by recombinant receptors $P2X_1$ and $P2X_2$ but is less potent than its derivative pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) (Evans *et al.*, 1995). P5P inhibits α,β-meATP-induced depolarization of rat superior cervical ganglion (Connolly, 1995).

5. PPADS. Although originally put forward as a P2X-selective antagonist, unfortunately it must now be accepted that PPADS is a non-selective (but non-universal) P2 receptor antagonist. PPADS is a slowly-equilibrating and slowly-reversible antagonist with pA_2 values of approximately 6 to 6.7 at endogenous $P2X_1$-like receptors in a variety of smooth muscle preparations (table 8; Lambrecht *et al.*, 1996; Ziganshin *et al.*, 1993, 1994b; Bültmann and Starke, 1994a; McLaren *et al.*, 1994; Windscheif *et al.*, 1994; Galligan *et al.*, 1995; Von Kügelgen *et al.*, 1995a; Eltze and Ullrich, 1996; Ralevic and Burnstock, 1996b; Usune *et al.*, 1996). It also blocks recombinant $P2X_1$, $P2X_2$, $P2X_3$, and $P2X_5$ receptors with IC_{50} values of 1 to 2.6 μM (Collo *et al.*, 1996). A lysine residue in receptors $P2X_1$, $P2X_2$, and $P2X_5$ (amino acid 249 in $P2X_1$) seems to be involved in the slowly reversible component of block by PPADS, probably involving formation of a Schiff's base (Buell *et al.*, 1996b). Rat recombinant $P2X_4$ and $P2X_6$ receptors are not blocked by PPADS (Buell *et al.*, 1996b; Collo *et al.*, 1996; Soto *et al.*, 1996a,b; Garcia-Guzman *et al.*, 1997a), but interestingly, the human homolog of the $P2X_4$ receptor is

blocked by PPADS with an IC_{50} of 28 μM (Garcia-Guzman *et al.*, 1997a). PPADS antagonizes depolarizations induced by α,β-meATP in rat superior cervical ganglion (Connolly, 1995).

PPADS generally blocks endogenous $P2Y_1$-like and recombinant $P2Y_1$ receptors coupled to PLC (Boyer *et al.*, 1994; Brown *et al.*, 1995; Charlton *et al.*, 1996a; Schachter *et al.*, 1996) but not those coupled to inhibition of adenylate cyclase (Boyer *et al.*, 1994; Webb *et al.*, 1996c). PPADS has been reported to be inactive at $P2Y_1$-like receptors in smooth muscle of rabbit mesenteric artery and endothelium of rabbit aorta (Ziganshin *et al.*, 1994b), but blocks those in rat duodenum, guinea-pig taenia coli (pA_2 values 5.1 and 5.3, respectively) (Windscheif *et al.*, 1995a), and rat mesenteric arterial bed (pA_2 value 6.0) (Ralevic and Burnstock, 1996b). PPADS blocks $P2Y_2$-like receptors in astrocytes from the dorsal horn of the spinal cord (IC_{50} approximately 0.9 μM) (Ho *et al.*, 1995) but not $P2Y_2$-like receptors on rat mesenteric arterial endothelium (Windscheif *et al.*, 1994; Ralevic and Burnstock, 1996a), or those on cultured bovine aortic endothelial cells (Brown *et al.*, 1995). PPADS antagonizes responses to UTP at the recombinant $P2Y_4$ receptor (IC_{50} value approximately 15 μM) (Communi *et al.*, 1996a). At high concentrations PPADS blocks $P2Y_{ADP}$ receptor-mediated ADP-induced platelet aggregation and inhibits ecto-nucleotidase activity (Windscheif *et al.*, 1995b; Chen *et al.*, 1996c). At concentrations greater than 10 μM, non-specific effects of PPADS have been reported involving inhibition of IP_3-induced $[Ca^{2+}]_i$ mobilization (Vigne *et al.*, 1996).

6. Iso-PPADS. An isomer of PPADS, pyridoxalphosphate-6-azophenyl-2',5'-disulfonic acid (iso-PPADS) is a slowly-equilibrating and slowly-reversible antagonist of responses at P2X receptors with similar potency to PPADS (Trezise *et al.*, 1994c) and competes for $[^3H]\alpha,\beta$-meATP binding sites in the rat vas deferens (Khakh *et al.*, 1994). Iso-PPADS blocks depolarizations evoked by α,β-meATP, but not those to UTP in rat superior cervical ganglion, but in contrast to PPADS also blocks depolarizations to muscarine (Connolly, 1995).

7. Reactive blue 2. The anthraquinone-sulfonic acid derivative reactive blue 2 (synonymous with cibacron blue) is a non-competitive P2 receptor antagonist which does not discriminate adequately between P2X and P2Y subtypes. In the vasculature, it has micromolar affinity and some selectivity for endothelial $P2Y_1$ and smooth muscle $P2Y_1$-like receptors versus other vascular P2X and P2Y receptors; however, selectivity versus the smooth muscle $P2X_1$-like receptor is low, and its use is limited by a narrow effective concentration range and time of exposure (Burnstock and Warland, 1987a; Hopwood and Burnstock, 1987; Houston *et al.*, 1987). Reactive blue 2 antagonism of P2Y receptors includes block of the recombinant $P2Y_6$ receptor (Chang *et al.*, 1995) and some endogenous $P2Y_2$-like and uridine nucleotide-specific receptors (Nakaoka and Yamashita, 1995; Chen *et al.*, 1996c). Reactive blue 2 blocks selectively contractile responses to ADPβS at a P2Y-like receptor, but enhances P2X receptor-mediated contractions to α,β-meATP and ATP in rat anococcygeus smooth muscle (Najbar *et al.*, 1996)

Reactive blue 2 also has been shown to block responses mediated by endogenous P2X receptors in adult rat superior cervical and nodose ganglia, and guinea-pig coeliac ganglion (Silinsky and Gerzanich, 1993; Connolly and Harrison, 1994; Khakh *et al.*, 1995a), rat vagus nerve (Trezise *et al.*, 1994c), urinary bladder and vas deferens (Choo, 1981; Bo *et al.*, 1994; Bültmann and Starke, 1994a; Suzuki and Kokubun, 1994), endogenous $P2X_7$-like receptors (McMillian *et al.*, 1993; Wiley *et al.*, 1993), and recombinant $P2X_2$ (Brake *et al.*, 1994) and $P2X_4$ (Bo *et al.*, 1995; Séguéla *et al.*, 1996) receptors. Thus, this compound does not discriminate adequately between P2X and P2Y receptors, although it may be useful in discriminating between subtypes of coexisting P2 receptors. Inhibition by reactive blue 2 of GABA and glutamate receptors (Motin and Bennett, 1995; Nakazawa *et al.*, 1995), and NMDA-gated ion channels (Peoples and Li, 1998) further advises caution in the use of this compound. Inhibition of ectoATPase activity by reactive blue 2 also has been reported (Stout and Kirley, 1995).

8. Reactive red. Reactive red is at least 350 times more potent than reactive blue 2 as a competitive antagonist at the $P2Y_1$-like receptor of guinea-pig taenia coli (K_d, 28 nM); however, it is only 15-fold selective versus the $P2X_1$-like receptor in rat vas deferens, and has ecto-nucleotidase activity (Bültmann and Starke, 1995). Its effects at other P2X and P2Y subtypes are largely unknown.

9. Trypan blue. Trypan blue blocks selectively (versus K^+ and noradrenaline) α,β-meATP-mediated contractions at the $P2X_1$-like receptor in rat vas deferens but is also an inhibitor of ADPβS-mediated relaxations via $P2Y_1$-like receptors in guinea-pig taenia coli and an inhibitor of ecto-nucleotidase activity (Bültmann *et al.*, 1994; Wittenburg *et al.*, 1996).

10. Evans blue. Evans blue blocks selectively responses to α,β-meATP in the rat vas deferens versus those mediated by ADPβS in the guinea-pig taenia coli, but potentiates contraction to ATP, ADP, and 2MeSATP in a manner attributable in part to ecto-nucleotidase inhibition; it also has non-specific potentiating effects (Bültmann and Starke, 1993; Bültmann *et al.*, 1995; Wittenburg *et al.*, 1996). The desmethyl derivative of Evans blue, NH01, is highly selective for the $P2X_1$-like receptor in vas deferens versus the $P2Y_1$-like receptor in guinea-pig taenia coli (K_d values 0.8 and > 100 μM, respectively), but is only moderately selective for the $P2X_1$ receptor versus inhibition of ecto-nucleotidase activity (Wittenburg *et al.*, 1996).

11. DIDS. The Cl^- transport blocker 4,4'-diisothiocyanatostilbene-2,2'-disulfonate (DIDS) is a noncompetitive, pseudo-irreversible antagonist of $P2X_1$-like recep-

tor-mediated contractions to α,β-meATP and of the purinergic component of the neurogenic contractile response in guinea-pig and rat vas deferens, and is selective versus the $P2Y_1$-like receptor of guinea-pig taenia coli (Fedan and Lamport, 1990; Bültmann and Starke, 1994b; Bültmann *et al.*, 1996a). However, it is nonselective versus inhibition of ecto-nucleotidase activity (Bültmann *et al.*, 1996a). DIDS discriminates between subtypes of P2X receptors, being a potent inhibitor of responses mediated at the $P2X_1$ receptor cloned from human bladder (IC_{50} 3 μM), but less than 40% effective at recombinant $P2X_2$ receptors from PC12 cells at concentrations of up to 300 μM (Evans *et al.*, 1995). DIDS blocks depolarization to α,β-meATP in rat superior cervical ganglia, but has no effect on depolarization to UTP or potassium, or hyperpolarization to adenosine (Connolly and Harrison, 1995a). DIDS and some analogs of DIDS also block endogenous $P2X_7$-like receptors (el-Moatassim and Dubyak, 1993; McMillian *et al.*, 1993; Soltoff *et al.*, 1993). DIDS, PPADS, and dextran sulfate discriminate between recombinant human $P2X_1$ and rat $P2X_2$ receptors in displacement of binding studies, having 7- to 33-fold higher affinity for $P2X_1$ receptors (Michel *et al.*, 1996).

12. Arylazidoaminopropionyl ATP ($ANAPP_3$). $ANAPP_3$, a photo-affinity analog of ATP, activates and desensitizes endogenous smooth muscle $P2X_1$-like receptors, irreversibly so after exposure to light, and selectively versus non-purine receptors (Hogaboom *et al.*, 1980; Fedan *et al.*, 1985; Venkova and Krier, 1993). Its effects at other P2X receptor subtypes have not been determined. However, $ANAPP_3$ also weakly antagonizes relaxations to ATP, ADP, and adenosine in the guinea-pig taenia coli (Westfall *et al.*, 1982).

13. 2-Alkylthio derivatives of ATP. 2-Alkylthio derivatives of ATP are potent $P2Y_1$ receptor antagonists: both base modifications, leading to 8-(6-aminohexylamino)-ATP and N-oxide ATP, and ribose modifications, leading to 2′,3′-isopropylidene-AMP, result in derivatives that display selectivity for endothelial $P2Y_1$-like receptors and are virtually inactive at smooth muscle $P2Y_1$-like and $P2X_1$-like receptors (Burnstock *et al.*, 1994).

14. 5′-p-Fluorosulfonyl benzoyladenosine. This is an irreversible inhibitor of ATP-induced Ba^{2+} influx via the $P2X_7$ receptor in human lymphocytes, although maximal inhibition does not exceed 90% (Wiley *et al.*, 1994).

IX. P2X Receptors

P2X receptors are ATP-gated ion channels which mediate rapid (within 10 ms) and selective permeability to cations (Na^+, K^+ and Ca^{2+})(Bean, 1992; Dubyak and el-Moatassim, 1993; North, 1996). This is appropriate given their distribution on excitable cells (smooth muscle cells, neurons, and glial cells) and role as mediators of fast excitatory neurotransmission to ATP in both the central and peripheral nervous systems. This contrasts with the slower onset of response (less than 100 ms) to ATP acting at metabotropic P_{2Y} receptors, which involves coupling to G proteins and second-messenger systems. Seven P2X receptor proteins ($P2X_1$ to $P2X_7$) have been cloned and the ion channels formed from homomeric association of the subunits when expressed in *Xenopus* oocytes or in mammalian cells have been functionally characterized and show distinct pharmacological profiles (table 9). The $P2X_7$ receptor is considered separately below (see Section X.) because it is functionally unique among P2X receptors in being able to act as a non-selective pore.

A. Structure

Structural features of P2X receptors have been predicted from the amino acid sequences of cloned P2X receptor subunits. It is important to bear in mind that the P2X proteins that have been cloned are receptor subunits, not actual receptors; a single 2 transmembrane subunit alone cannot form an ion channel. The proteins have 379 to 472 amino acids and are believed to insert into the cell membrane to form a pore comprising two hydrophobic transmembrane domains, with much of the protein occuring extracellularly as an intervening hydrophilic loop (fig. 8). The overall structure of the receptor most closely resembles that of amiloride-sensitive epithelial Na^+ channels. The putative extracellular loop of cloned receptors $P2X_1$ to $P2X_7$ has 10 conserved cysteine residues, 14 conserved glycine residues and 2 to 6 potential N-linked glycosylation sites. It is believed that disulfide bridges may form the structural constraints needed to couple the ATP-binding site to the ion pore. Most of the conserved regions are in the extracellular loop, with the transmembrane domains being less well-conserved.

The quaternary structures of classical ligand-gated channels, for example, those of the nicotinic ACh receptor and the epithelial Na^+ channel, generally take the form of heteromeric complexes of structurally related subunits. P2X receptors are believed to complex in a similar way in biological tissues. Their subunit stoichiometry is unknown, but may involve three subunits (or multiples of three subunits) based on SDS polyacrylamide gel electrophoresis estimates of the relative molecular mass of the recombinant $P2X_1$ and $P2X_3$ receptors determined under non-denaturing conditions (Nicke *et al.*, 1998).

The pharmacological properties of endogenous P2X receptors in smooth muscle and PC12 cells correlate well with those of the recombinant receptors cloned from these tissues, $P2X_1$ and $P2X_2$ receptors, respectively; both native and recombinant $P2X_1$ receptors are sensitive to α,β-meATP and rapidly desensitize, whereas $P2X_2$ receptors are insensitive to α,β-meATP and do not desensitize. A good correlation is also seen between the properties of endogenous P2X receptors in neonatal dorsal root ganglion and the recombinant $P2X_3$ receptor (cloned from and expressed predominantly or exclu-

TABLE 9
Cloned P2X receptors

Receptor	Number of amino acids	cDNA library source	Agonist activity	References
$P2X_1$	399	Human urinary bladder	ATP > α,β-meATP	Valera *et al.*, 1995; Longhurst *et al.*, 1996
		Rat vas deferens	2MeSATP > ATP > α,β-meATP	Valera *et al.*, 1994
		Mouse urinary bladder	—	Valera *et al.*, 1996
$P2X_2$	472	Rat PC12 cells	2MeSATP > ATP; α,β-meATP inactive	Brake *et al.*, 1994
$P2X_{2(b)}$[a]	401	Rat cerebellum	2MeSATP = ATP = α,β-meATP	Brändle *et al.*, 1997; Simon *et al.*, 1997
$P2X_3$	397	Human heart, spinal cord	2MeSATP > ATP > α,β-meATP	Garcia-Guzman *et al.*, 1997b
		Rat DRG cells	2MeSATP > ATP > α,β-meATP > UTP	Chen *et al.*, 1995a
		Rat DRG cells	ATP > 2MeSATP > α,β-meATP	Lewis *et al.*, 1995
$P2X_4$	388	Human brain	ATP ≫ 2MeSATP ≥ CTP > α,β-meATP	Garcia-Guzman *et al.*, 1997a
		Rat brain	ATP ≫ 2MeSATP ≥ CTP > α,β-meATP	Soto *et al.*, 1996a
		Rat brain	ATP > 2MeSATP ≫ α,β-meATP	Séguéla *et al.*, 1996
		Rat hippocampus	ATP > 2MeSATP ≫ α,β-meATP	Bo *et al.*, 1995
		Rat SCG	ATP; α,β-meATP inactive	Buell *et al.*, 1996b
		Rat pancreatic islet	ATP, ADP, 2MeSATP ≫ α,β-meATP	Wang *et al.*, 1996
$P2X_5$	417	Rat ganglia	ATP > 2MeSATP > ADP α,β-meATP inactive	Collo *et al.*, 1996
	455	Rat heart	ATP > 2MeSATP > ADP	Garcia-Guzman *et al.*, 1996
$P2X_6$	379	Rat superior cervical ganglion	ATP > 2MeSATP > ADP; α,β-meATP inactive	Collo *et al.*, 1996
		Rat brain	—	Soto *et al.*, 1996b
$P2X_7$	595	Mouse macrophage	BzATP > ATP > UTP ATP > UTP > BzATP	Nuttle *et al.*, 1993
		Rat macrophage and brain	BzATP > ATP > 2MeSATP > ADP; UTP inactive	Surprenant *et al.*, 1996
	595	Human monocytes	BzATP > ATP	Rassendren *et al.*, 1997

[a] Splice variant, also termed $P2X_{2\text{-}2}$.

sively in sensory neurons); both are sensitive to α,β-meATP and rapidly desensitize (Evans and Surprenant, 1996). Thus, there is good reason to believe that the native P2X receptors in these tissues are predominantly homomers formed by the association of a single type of subunit.

However, this is not always the case. ATP-gated currents at endogenous P2X receptors in rat nodose neurons are mimicked by α,β-meATP and do not desensitize (Lewis *et al.*, 1995), a pharmacological profile that does not correspond to any of the homomeric P2X receptors cloned to date; all are expressed in sensory ganglia except $P2X_7$. Although $P2X_3$ is expressed preferentially in sensory neurons, currents evoked by ATP and α,β-meATP at the recombinant $P2X_3$ receptor rapidly desensitize. However, when $P2X_3$ is coexpressed in HEK293 cells with $P2X_2$ (but not with other subtypes), a nondesensitizing response to ATP is observed which mimicks that seen in rat nodose neurons and which cannot be explained by additive effects of the two homomeric channels (Lewis *et al.*, 1995). It was suggested that a new heteromeric receptor, $P2X_2P2X_3$, is formed from the $P2X_3$ and $P2X_2$ subunits (Lewis *et al.*, 1995). This hypothesis is supported by the observation of a high level of colocalization of $P2X_2$- and $P2X_3$-immunoreactivity in rat nodose and dorsal root ganglia (Vulchanova *et al.*, 1997). Direct evidence for the formation of a $P2X_2P2X_3$ heteromer comes from a study showing that in cells coinfected with $P2X_2$ and $P2X_3$ receptors, the two proteins can be cross-immunoprecipitated with antibodies specific for either of the epitope tags introduced at the C terminal of the proteins (Radford *et al.*, 1997). Electrophysiological studies showing sensitivity to α,β-meATP and a slowly desensitizing response is consistent with formation of heteromeric receptors because this is distinct from responses mediated by homomeric $P2X_2$ and $P2X_3$ receptors (Radford *et al.*, 1997).

Further evidence for the existence of $P2X_2P2X_3$ heteromers in sensory neurons is suggested by electrophysiological studies in cultured neurons of adult rat dorsal root (Robertson *et al.*, 1996) and trigeminal ganglion neurons (Cook *et al.*, 1997). However, heterogeneity within populations of sensory neurons has been identified in the form of single labeling for $P2X_2$ or $P2X_3$ of some rat nodose and dorsal root neurons (possibly coexisting with other P2X proteins) (Vulchanova *et al.*, 1997), and by the demonstration of two types of inward current to ATP (transient and slowly desensitizing) in tooth-pulp nociceptors (Cook and McCleskey, 1997). This raises interesting questions about the patterns of P2X receptor subtype expression and the physiological properties of different neurons.

The likely formation of $P2X_2P2X_3$ heteromers in sensory neurons has important implications for the subunit organization of P2X receptors in other biological tissues, because the different P2X proteins have widespread and

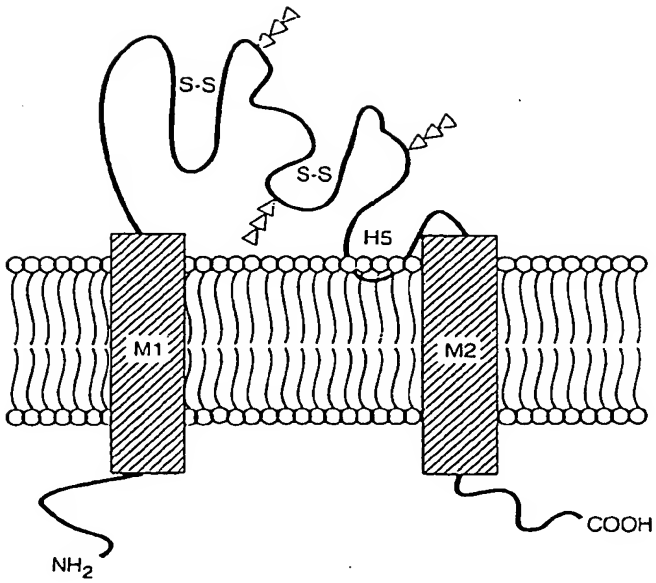

FIG. 8. Diagram depicting a proposed transmembrane topology for $P2X_2$ protein showing both N- and C-terminals in the cytoplasm. Two putative membrane spanning segments (M_1 and M_2) traverse the lipid bilayer of the plasma membrane and are connected by a hydrophilic segment of 270 amino acids. This putative extracellular domain is shown containing two disulfide-bonded loops (S-S) and three N-linked glycosyl chains (triangles). The $P2X_2$ cDNA was sequenced on both strands using Sequanase. (From Brake *et al.*, 1994).

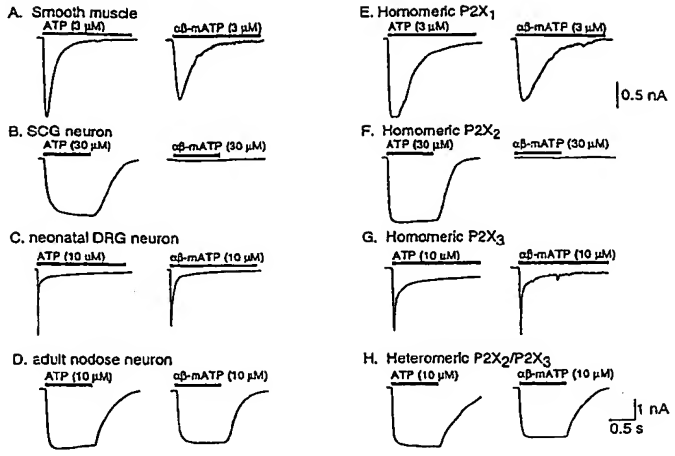

FIG. 9. Examples of ATP-gated currents evoked in native cells (A-D) and in HEK293 cells expressing homomeric (E-G) or heteromeric (H) P2X receptors. Bars above each trace refer to the duration of agonist application. All recordings are at holding potential of −70 mV. Traces shown in C from neonatal dorsal root ganglion neurons are unpublished records kindly supplied by M. Rae, S. Robertson, E. Rowan, and C. Kennedy, University of Strathclyde; all other traces from authors unpublished records. (From Evans and Surprenant, 1996.)

overlapping distributions. However, it seems that not all combinations are possible; for example, cotransfected $P2X_1$ and $P2X_2$ subunits do not combine to form heteromeric receptors (Surprenant, 1996). Figure 9 shows examples of ATP-gated currents in native cells and how these correlate with recombinant P2X receptors.

Alternative splicing of P2X pre-messenger RNA has been shown for the $P2X_2$ receptor (Brändle *et al.*, 1997; Simon *et al.*, 1997). The splice variant exhibits a different pharmacology to the native receptor, suggesting that there may be heterogeneity in responses of tissues expressing the different proteins.

B. Cloned P2X Receptors

1. $P2X_1$ receptor. The $P2X_1$ receptor has been cloned from rat vas deferens and human and mouse urinary bladder (Valera *et al.*, 1994, 1995, 1996) (table 9). The recombinant receptor is activated by 2MeSATP $\geq$ ATP > α,β-meATP $\gg$ ADP, and inward currents evoked by these compounds are reversibly blocked by suramin and PPADS (Valera *et al.*, 1994). The receptor desensitizes very rapidly (in hundreds of milliseconds).

$P2X_1$ receptor mRNA is expressed in urinary bladder, smooth muscle layers of small arteries and arterioles, and vas deferens, with lower levels in lung and spleen (Valera *et al.*, 1994; Collo *et al.*, 1996). $P2X_1$ receptor mRNA is also expressed in dorsal root ganglia, trigeminal ganglia, coeliac ganglia, spinal cord, and rat brain (Valera *et al.*, 1994; Webb *et al.*, 1995; Collo *et al.*, 1996).

The $P2X_1$ receptor seems to be the most significant P2X subtype in vascular smooth muscle, although $P2X_4$ receptors may also be expressed (Soto *et al.*, 1996a). The similar pharmacological profiles and desensitization of the recombinant $P2X_1$ receptor and its native counterpart is consistent with the concept that the vascular smooth muscle P2X receptor is a $P2X_1$ receptor homomer. ATP-gated ion channels in platelets and megakaryocytes have a similar pharmacology to the recombinant $P2X_1$ receptor, which has led to the suggestion that these ion channels are $P2X_1$ receptors (Somasundaram and Mahaut-Smith, 1994; MacKenzie *et al.*, 1996).

2. $P2X_2$ receptor. The $P2X_2$ receptor first cloned from rat pheochromocytoma PC12 cells (originally called P2XR1) (Brake *et al.*, 1994) displays only 41% amino acid homology with the rat vas deferens $P2X_1$ receptor. At the recombinant $P2X_2$ receptor ATP, adenosine 5′-O-(3-thiotriphosphate) (ATPγS) and 2MeSATP are approximately equipotent at eliciting non-selective inward cation currents, whereas α,β-meATP and β,γ-meATP are inactive as agonists or antagonists (Brake *et al.*, 1994). This receptor undergoes little or no desensitization. It also differs from the $P2X_1$ receptor in that it is less permeable to Ca^{2+} and shows much higher sensitivity to inhibition by extracellular Ca^{2+} (Evans *et al.*, 1996).

$P2X_2$ receptor mRNA is distributed in bladder, brain, spinal cord, superior cervical ganglia, adrenal medulla, intestine, and vas deferens, with highest levels found in the pituitary gland and vas deferens (Brake *et al.*, 1994). Distinct but restricted patterns of distribution of $P2X_2$ mRNA have been described within rat brain (Collo *et al.*, 1996). $P2X_2$ receptor mRNA is the only P2X mRNA

observed in the adrenal medulla (Collo *et al.*, 1996). $P2X_2$ mRNA is absent from skeletal muscle, and several organs including heart, liver, kidney, lung, and spleen. Immunohistochemical detection shows a widespread distribution of the $P2X_2$ receptor in brain and spinal cord (Vulchanova *et al.*, 1996). The pharmacological profile of the P2X response in PC12 cells, namely insensitivity to α,β-meATP and lack of desensitization, is consistent with the concept that this is an endogenous counterpart of the $P2X_2$ receptor.

Sequence homology (about 40%) between $P2X_2$ and a partial cDNA called RP-2 encoding for a protein activated in thymocytes undergoing programmed cell death, has led to the suggestion that RP-2 may encode an ion channel subunit activated by ATP released during apoptosis (Brake *et al.*, 1994).

A splice variant of a $P2X_2$ receptor has been isolated from rat cerebellum and characterized pharmacologically (Brändle *et al.*, 1997; Simon *et al.*, 1997). The protein, termed $P2X_{2(b)}$ or $P2X_{2-2}$, has a 69 amino acid deletion of the carboxyl-terminal, shows a similar distribution in the rat central and peripheral nervous system as the original $P2X_2$ receptor (distinguished by the terminology $P2X_{2(a)}$), and forms a homomeric receptor mediating inward currents to ATP (Brändle *et al.*, 1997; Simon *et al.*, 1997). Although the $P2X_{2(b)}$ receptor was equally sensitive to agonists as the $P2X_{2(a)}$ receptor, it showed significantly lower antagonist sensitivity and a faster rate of desensitization. Two other splice variants were also identified, and designated $p2X_{2(c)}$ and $p2X_{2(d)}$ to indicate that their functional significance remains to be determined (Simon *et al.*, 1997).

A truncated form of the $P2X_2$ receptor (360 amino acids compared with the 472 of $P2X_2$), $P2X_{2-1}$ (originally called P2xR1), has been isolated from the pituitary gland and secretory epithelial tissue of rat cochlea (Housley *et al.*, 1995).

3. $P2X_3$ receptor. The $P2X_3$ receptor cloned from rat dorsal root ganglion (Chen *et al.*, 1995a; Lewis *et al.*, 1995) shows only 43% amino acid sequence homology with the $P2X_1$ receptor and 47% identity to the $P2X_2$ receptor. The $P2X_3$ receptor is activated by agonists with a potency order of 2MeSATP $\gg$ ATP $>$ α,β-meATP and undergoes rapid desensitization (in less than 100 ms).

The $P2X_3$ receptor has a very restricted distribution; it is expressed only by a subset of sensory neurons (trigeminal, nodose, and dorsal root ganglia), and is absent from sympathetic, enteric and central nervous system neurons, and smooth muscle (Chen *et al.*, 1995a; Lewis *et al.*, 1995; Collo *et al.*, 1996). All of the other cloned P2X receptors also have been localized in sensory neurons. The human $P2X_3$ receptor transcript is limited to spinal cord and heart (Garcia-Guzman *et al.*, 1997b). Interestingly, whereas the homomeric $P2X_3$ receptor accounts for rapidly desensitizing currents to ATP and α,β-meATP in neonatal sensory neurons (Krishtal *et al.*, 1988a, 1988b; Li *et al.*, 1993; Robertson *et al.*, 1996), a heteromeric $P2X_2P2X_3$ receptor seems to account for the nondesensitizing response in adult sensory neurons (Lewis *et al.*, 1995), suggesting that there may be differential expression of P2X subunits in sensory neurons in development.

4. $P2X_4$ receptor. The $P2X_4$ receptor protein has been cloned from rat hippocampus (Bo *et al.*, 1995), DRG cells (Buell *et al.*, 1996b), rat (Séguéla *et al.*, 1996; Garcia-Guzman *et al.*, 1997a) and human brain (Soto *et al.*, 1996a; Garcia-Guzman *et al.*, 1997a), as well as rat endocrine tissue (Wang *et al.*, 1996). The P2X receptor cloned from rat brain by Séguéla *et al.* (1996) was refered to as P_{2x3} in their paper, but a comparison of the receptor sequence with known subtypes identifies it as $P2X_4$. A sequence homology of 87% between the human and rat $P2X_4$ receptors is sufficiently different to produce subtle differences in antagonist binding and desensitization. The recombinant $P2X_4$ receptor is most potently activated by 2MeSATP, but α,β-meATP is weak or inactive (Bo *et al.*, 1995; Séguéla *et al.*, 1996). $P2X_4$ is relatively insensitive to the antagonists suramin and PPADS; high concentrations (>100 μM) are required to block ATP-evoked currents (Bo *et al.*, 1995; Séguéla *et al.*, 1996), although the human receptor shows a higher sensitivity for suramin and PPADS (Garcia-Guzman *et al.*, 1997a). A lysine residue present in the $P2X_1$ and $P2X_2$ receptors, but absent in the $P2X_4$ receptor, is critical for the binding of antagonists but not agonists (Buell *et al.*, 1996a). The $P2X_4$ receptor does not desensitize rapidly, although reversible rundown of the current occurs during prolonged exposure to ATP (Séguéla *et al.*, 1996). More rapid desensitization of the human $P2X_4$ receptor (Garcia-Guzman *et al.*, 1997a) compared with the rat $P2X_4$ receptor (Buell *et al.*, 1996a) has been described. $P2X_4$ ATP-gated currents are potentiated by coapplication of Zn^{2+} (Séguéla *et al.*, 1996; Garcia-Guzman *et al.*, 1997a).

$P2X_4$ receptor mRNA is expressed in brain, spinal cord, sensory ganglia, superior cervical ganglion, lung, bronchial epithelium, thymus, bladder, acinar cells of the salivary gland, adrenal gland, testis, and vas deferens (Bo *et al.*, 1995; Buell *et al.*, 1996b; Collo *et al.*, 1996; Séguéla *et al.*, 1996). Within the brain and spinal cord, the distribution of $P2X_4$ mRNA is very similar to, but not identical with, that of the $P2X_6$ receptor (Collo *et al.*, 1996). $P2X_4$ receptor mRNA is unique in that it is the only type expressed by acinar cells of the salivary gland (Collo *et al.*, 1996).

5. $P2X_5$ receptor. This P2X receptor was first cloned from rat coeliac ganglia (Collo *et al.*, 1996). Human homologs of the $P2X_5$ receptor have tentatively been identified (Tokuyama *et al.*, 1996a, 1996b). Rapid inward currents are activated by ATP $>$ 2MeSATP $>$ ADP, whereas α,β-meATP is ineffective as an agonist. The receptor does not readily desensitize. Currents are readily inhibited by suramin and PPADS. In situ hybridization shows $P2X_5$ mRNA in motoneurons of the ven-

tral horn of the cervical spinal cord, and in neurons in the trigeminal and dorsal root ganglia. With the exception of the mesencephalic nucleus of the trigeminal nerve, the brain does not express $P2X_5$ mRNA (Collo *et al.*, 1996). Appropriately, functional studies have identified P2X receptors in rat trigeminal mesencephalic nucleus neurons with a profile most similar to that of $P2X_5$ receptors (Khakh *et al.*, 1997)

6. $P2X_6$ receptor. This clone was isolated from a rat superior cervical ganglion cDNA library (Collo *et al.*, 1996). Rapid currents are mediated by ATP > 2MeSATP > ADP, but α,β-meATP has no effect. Currents are only partially inhibited by suramin or PPADS. $P2X_6$ mRNA is heavily expressed in the CNS, with heaviest staining in cerebellar Purkinje cells and ependyma (Collo *et al.*, 1996). Staining is also detected in the cervical spinal cord, notably in spinal motoneurons of lamina IX, and the superficial dorsal horn neurons of lamina II. $P2X_6$ mRNA is also present in trigeminal, dorsal root, and coeliac ganglia; and in gland cells of the uterus, granulosa cells of the ovary, and bronchial epithelia, but is absent from salivary epithelia, adrenal medulla, and bladder smooth muscle (Collo *et al.*, 1996).

7. $P2X_7$ receptor. This receptor is considered in detail in Section X.

C. Signal Transduction Mechanisms

P2X receptors mediate the rapid (onset within 10 ms) non-selective passage of cations (Na^+, K^+, Ca^{2+}) across the cell membrane resulting in an increase in intracellular Ca^{2+} and depolarization (Bean, 1992; Dubyak and el-Moatassim, 1993). The direct flux of extracellular Ca^{2+} through the channel constitutes a significant source of the increase in intracellular Ca^{2+}. However, membrane depolarization leads to the secondary activation of voltage-dependent Ca^{2+} channels, which probably make the primary contribution to Ca^{2+} influx and to the increase in intracellular Ca^{2+}. Because this transduction mechanism does not depend on the production and diffusion of second-messengers within the cytosol or cell membrane, the response time is very rapid, and appropriately plays an important role in fast neuronal signaling and regulation of muscle contractility. P2X channels often show considerable current fluctuation, or "flickery bursts," in the open state that may represent unresolved closures or rapid transition between states (Evans and Surprenant, 1996). Selectivity for Ca^{2+} permeability between P2X receptors on sensory versus autonomic nerves and smooth muscle has been suggested, but the patterns are not entirely clear (see Evans and Surprenant, 1996). The kinetics of ATP-gated currents have been reviewed (Surprenant, 1996).

Cations can modulate ATP-activated currents in native and endogenous P2X receptors. Mg^{2+} and Ca^{2+} generally inhibit P2X receptor currents, probably by decreasing the affinity of the ATP binding site by an allosteric change in the receptor (Honoré *et al.*, 1989; Nakazawa *et al.*, 1990; Li *et al.*, 1997a). However, an increase in the transient ATP response (but not the slowly-desensitizing ATP response) has been observed when Ca^{2+} replaces Na^+ in the extracellular solution in rat trigeminal sensory neurons (Cook and McCleskey, 1997). Interestingly, the recombinant $P2X_2$ receptor seems to be more susceptible than the $P2X_1$ receptor to inhibition by increases in extracellular Ca^{2+} (Evans *et al.*, 1996). Allosteric interactions may also be responsible for the ability of monovalent cations to negatively modulate binding to recombinant $P2X_4$ receptors (Michel *et al.*, 1997), and trivalent cations to negatively modulate the binding site of recombinant $P2X_1$ and $P2X_2$ receptors and the endogenous receptor of PC12 cells (Nakazawa *et al.*, 1997).

Zn^{2+} potentiates the cation conductance induced by ATP at most P2X receptors, including those in rat superior cervical ganglion (Cloues *et al.*, 1993; Cloues, 1995), nodose and coeliac ganglion neurons (Li *et al.*, 1993, 1996), PC12 cells (Koizumi *et al.*, 1995a), and recombinant $P2X_1$ (Brake *et al.*, 1994) and $P2X_4$ receptors (Séguéla *et al.*, 1996). The $P2X_7$ receptor is an exception in this respect because it is inhibited by Zn^{2+} and Cu^{2+} (Virginio *et al.*, 1997). Ni^{2+} enhances ATP-activated currents in rat superior cervical ganglia (Cloues *et al.*, 1993) and Cd^{2+} potentiates ATP-evoked inward currents and dopamine release in rat phaeochromocytoma cells (Ikeda *et al.*, 1996).

Modulation of the affinity of the ATP-binding site occurs by extracellular protons; acid pH causes an increase, and alkaline pH causes a decrease in currents, as shown for the recombinant $P2X_2$ receptor and endogenous P2X receptors in rat dorsal root and nodose ganglion cells (King *et al.*, 1996b; Li *et al.*, 1996, 1997b; Wildman *et al.*, 1997). This may be particularly significant for P2X receptor-mediated signaling in pathophysiological conditions where injury or inflammation can profoundly alter extracellular pH.

D. Desensitization

P2X receptors can be divided into two broad groups according to whether they desensitize rapidly, that is, within 100 to 300 ms, or slowly if at all (table 10). This subdivision hinges critically on the time to desensitization; "rapid" desensitization should not be confused with desensitization which occurs over a few seconds, and thus is a phenomenon which is difficult to identify in other than studies of single channel activity. As a general rule, all rapidly desensitizing P2X receptors are activated by α,β-meATP as well as by 2MeSATP and ATP. These include: recombinant $P2X_1$ and $P2X_3$ receptors; their endogenous counterparts, namely $P2X_1$-like receptors of smooth muscle (with some exceptions, indicated below); $P2X_1$-like receptors of promyelocyte HL60 cells (Buell *et al.*, 1996b); and platelets (MacKenzie *et al.*, 1996) and $P2X_3$-like receptors of neonatal sensory neurons (dorsal root ganglion and nodose ganglion)

TABLE 10
Distinguishing pharmacological characteristics of P2 receptors

P2X receptors	Desensitization	α,β-meATP sensitivity	PPADS sensitivity	Suramin sensitivity
$P2X_1$	Rapid	Yes	Yes	Yes
$P2X_2$	Slow	—	Yes	Yes
$P2X_3$	Rapid	Yes	Yes	Yes
$P2X_4$	Slow	—	—	—
$P2X_5$	Slow	—	Yes	Yes
$P2X_6$	Slow	—	—	—
$P2X_7$ (P_{2Z})	Slow	—	N.D.	Yes
$P2X_2P2X_3$	Slow	Yes	N.D.	N.D.

P2Y receptors	Agonist sensitivity				
	2MeSATP	ATP	UTP	ADP	UDP
$P2Y_1$	Yes	Yes	—	Yes	—
$P2Y_2$	—	Yes	Yes	—	—
p2y3	—	—	Yes	Yes	Yes
$P2Y_4$	—	Yes[a]	Yes	—	—
$P2Y_6$	—	—	—	—	Yes
$P2Y_{11}$	Yes	Yes	—	—	—
$P2Y_{ADP}$	—	—[b]	—	Yes	—
Endogenous uridine nucleotide-specific	—	—	Yes	—	Yes

—, weak or inactive; N.D., not determined.
[a] Rat, but not human. $P2Y_4$ receptor is sensitive to ATP = UTP.
[b] ATP is a competitive antagonist.
Lower case is used to designate the p2y3 receptor in recognition that it is a nonmammalian (chick) receptor and may be the homolog of the mammalian $P2Y_6$ receptor.

(Krishtal *et al.*, 1988a,b; Li *et al.*, 1993; Robertson *et al.*, 1996). Desensitization of $P2X_3$-like receptors of neonatal sensory neurons, but not $P2X_1$-like receptors of smooth muscle, is concentration-dependent (Evans and Surprenant, 1996; Robertson *et al.*, 1996). Desensitization will clearly serve to terminate the purinergic response even though ATP release may still be ongoing, but exactly why this is more important in some tissues remains to be determined.

P2X receptors which do not desensitize rapidly, desensitize slowly or not at all. These "non-desensitizing" P2X receptors are defined as receptors for which the currents are maintained for at least a few seconds in the continuous presence of agonist. Non-desensitizing P2X receptors can be further subdivided into two groups: 1) those that are sensitive to α,β-meATP, and 2) those that are insensitive or only weakly sensitive to α,β-meATP (Evans and Surprenant, 1996). Non-desensitizing α,β-meATP-sensitive P2X receptors are those in adult sensory ganglia (nodose and dorsal root ganglion) (Krishtal *et al.*, 1988a, 1988b; Li *et al.*, 1993; Khakh *et al.*, 1995a; Wright and Li, 1995), and guinea-pig coeliac ganglion (Evans *et al.*, 1992; Khakh *et al.*, 1995a). It has been suggested that these receptors may be heteromers of $P2X_2$ and $P2X_3$ subunits ($P2X_2P2X_3$ receptors) (Lewis *et al.*, 1995) (fig. 9). Non-desensitizing α,β-meATP-sensitive responses have also been shown in some smooth muscle, namely in the arterial vasculature of human placenta (Dobronyi *et al.*, 1997; Ralevic *et al.*, 1997), and intestine of the three-spined stickleback *Gasterosteus aculeatus* L (Knight and Burnstock, 1993), and similarly may be caused by actions at P2X heteromers. Non-desensitizing α,β-meATP-sensitive P2X receptors have also been described in the CNS, on rat locus coeruleus neurons (Tschöpl *et al.*, 1992; Shen and North, 1993), and some rostral ventrolateral medulla neurons (Ralevic *et al.*, 1996).

Non-desensitizing α,β-meATP-insensitive P2X receptors are cloned $P2X_2$, $P2X_4$, $P2X_5$, and $P2X_6$ receptors (table 10a), as well as native P2X receptors on most autonomic neurons, including rat superior cervical ganglia (Cloues *et al.*, 1993; Nakazawa and Inoue, 1993; Khakh *et al.*, 1995a), guinea-pig submucosal enteric neurons (Barajas-Lopez *et al.*, 1994), PC12 cells (Nakazawa *et al.*, 1990; Nakazawa and Hess, 1993; Kim and Rabin, 1994), rat cardiac parasympathetic ganglia (Fieber and Adams, 1991), and chick ciliary ganglion neurons (Abe *et al.*, 1995). Non-desensitizing α,β-meATP-insensitive receptors have also been described in the CNS in nucleus tractus solitarius neurons (Ueno *et al.*, 1992; Nabekura *et al.*, 1995) and trigeminal mesencephalic nucleus neurons (Khakh *et al.*, 1997); these may correspond to $P2X_4$, $P2X_5$, or $P2X_6$ receptors, or to combinations of these subunits, given the rich expression of these proteins in the brain. ATP-gated α,β-meATP-insensitive currents in myometrial smooth muscle cells from pregnant rats have been reported to be resistant to desensitization (Honoré *et al.*, 1989).

The mechanism of P2X receptor desensitization is not well understood. For the rapidly desensitizing $P2X_1$ receptor, this may involve the hydrophobic domains of the receptor because transfer to the $P2X_2$ receptor of both of the hydrophobic domains, but not the extracellular loop, of the $P2X_1$ receptor changes the phenotype of the $P2X_2$ receptor from non-desensitizing to rapidly-desensitizing (Werner *et al.*, 1996). Amino acid deletions of the carboxyl terminal of the $P2X_2$ receptor produces splice variants that desensitize more rapidly than the original receptor (Brändle *et al.*, 1997; Simon *et al.*, 1997). On the other hand, the N-terminal region of the receptor has

been suggested to be important in desensitization of the $P2X_3$ receptor (King *et al.*, 1997). Desensitization of the $P2X_3$ receptor seems to involve the activation of calcineurin through the entry of extracellular calcium (King *et al.*, 1997).

E. Agonists and Antagonists

There are no universal or subtype-selective P2X receptor agonists. ATP and diadenosine polyphosphates with a phosphate chain length greater than or equal to three are naturally-occuring agonists at P2X receptors (Hoyle *et al.*, 1989; Hoyle, 1990; Bo *et al.*, 1994; Schlüter *et al.*, 1994; Bailey and Hourani, 1995; Ralevic *et al.*, 1995a; Usune *et al.*, 1996). The greater potency of the longer chain diadenosine polyphosphates (Ap_4A-Ap_6A) compared with ATP at endogenous $P2X_1$-like receptors may be caused by their greater resistance to breakdown (Hoyle, 1990; Ogilvie, 1992; Ralevic *et al.*, 1995a). UTP is a weak agonist of $P2X_3$ receptors (Chen *et al.*, 1995a; Robertson *et al.*, 1996) and may interact with $P2X_1$-like receptors in rat urinary bladder (Hashimoto and Kokubun, 1995) as well as mouse vas deferens (Von Kügelgen *et al.*, 1990).

In physiological solution, Ca^{2+} and Mg^{2+} ions form complexes with the free acid ATP^{4-}, such that the solution contains a mixture of ATP^{4-}, $MgATP^{2-}$, and $CaATP^{2-}$ (together with lower concentrations of the species variants $MgHATP^-$, $CaHATP^-$, and Ca_2ATP). Under physiological conditions, ATP^{4-} is a minor component of the total ATP concentration (approximately 1 to 10% depending on temperature, pH, and divalent cation concentration). The concentration of ATP^{4-} decreases with increasing cation concentration and with acidic pH (that results in conversion of ATP^{4-} to $HATP^{3-}$, which has proved useful in studies aimed at investigating the identity of the active form of ATP). Cockroft and Gomperts (1980) raised the question of which was the active form of ATP with their suggestion that ATP^{4-} causes an increase in mast cell plasma membrane permeability. It has since been shown that this form of the ligand is likely to be responsible for pore-forming actions in mast cells, macrophages, and lymphocytes as well as a number of other cell types expressing a receptor termed the P_{2Z} or $P2X_7$ receptor. Addition of Mg^{2+} forms the inactive species $MaATP^{2-}$ and thereby reduces the concentration of ATP^{4-}, rapidly closing the cation channel (Greenberg *et al.*, 1988; el-Moatassim and Dubyak, 1993; Gargett *et al.*, 1996; Lin and Lee, 1996). Similarly, 3′-O-(4-benzoyl)benzoyl ATP ($BzATP^{4-}$), and not the complex $MgBzATP^{2-}$, seems to be the active species in P_{2Z} or $P2X_7$-mediated pore formation.

The idea that ATP^{4-} is the active form of ATP has been extended to P2X receptors other than the P_{2Z} or $P2X_7$ receptor. Hence, ATP^{4-} has been suggested to be the ligand that activates P2X receptors in guinea-pig vas deferens smooth muscle (Fedan *et al.*, 1990), rat parotid acinar cells (McMillian *et al.*, 1993), and PC12 cells (Kim and Rabin, 1994; Choi and Kim, 1996); it also mediates ATP-gated currents in pregnant rat myometrial smooth muscle cells (Honoré *et al.*, 1989). The P2X receptors expressed by these tissues do not form nonspecific membrane pores. In these studies, suggestion of a role for ATP^{4-} as the active ligand is based primarily on the fact that responses are inhibited by elevation of extracellular Mg^{2+} or other cations which chelate with ATP, and because responses correlate well with the calculated ATP^{4-} concentration and not with the total ATP concentration or with the concentration of Mg^{2+} in solution. However, this alone does not seem to be sufficient evidence in light of more recent studies which show that divalent cations can influence agonist potency by effects other than by changes in the relative concentrations of the ATP species in solution.

It is now apparent that interpretation of the effects of removal of Mg^{2+} and Ca^{2+} from solution on agonist potency is complicated by additional inhibition of ecto-nucleotidase activity, disinhibition of single channel conductance of P2X receptors, and possibly membrane depolarization. These effects seem to have a greater influence on the end response than does a shift in the concentration of the active species of ATP. Inhibition of ecto-nucleotidase activity seems to be the overriding effect of Ca^{2+} and Mg^{2+} removal on agonist potency in the rat isolated vagus nerve, where the potency of responses to ATP and 2MeSATP was increased, but that of the stable analog α,β-meATP was unchanged (Trezise *et al.*, 1994a). Studies on single channel conductance of native P2 receptors in rat nodose ganglion, PC12 cells, and recombinant $P2X_1$ and $P2X_2$ receptors, in which consideration of ecto-nucleotidase activity is effectively bypassed in conditions of concentration clamp, have confirmed that raising Ca^{2+} or Mg^{2+} decreases the potency of ATP (Nakazawa and Hess, 1993; Evans *et al.*, 1996; Li *et al.*, 1997a; Virginio *et al.*, 1997). However, the mechanism seems to involve a decrease in the affinity of the agonist binding site by allosteric effects on the receptor (although direct cation block of the channels is also possible) (Nakazawa and Hess, 1993; Evans *et al.*, 1996; Li *et al.*, 1997a). The fact that recombinant $P2X_2$ receptors show a higher sensitivity than $P2X_1$ receptors to inhibition by extracellular Ca^{2+} (Evans *et al.*, 1996) is further consistent with the hypothesis that cation modulation of P2X receptors is due to changes occuring at the level of the receptor, and can be influenced by the intrinsic properties of that receptor, rather than a change in the relative concentrations of ATP species in the extracellular solution. Because of these complicating factors, the identity of the active species of ATP acting at P2X receptors is currently unclear.

α,β-MeATP is an agonist at recombinant $P2X_1$, $P2X_3$, and heteromeric $P2X_2P2X_3$ receptors; endogenous $P2X_1$-like receptors in smooth muscle, platelets, and HL60 cells; $P2X_3$-like receptors in neonatal nodose and dorsal root ganglia; and P2X receptors in guinea-pig coeliac

TABLE 11
P2 receptors in the central nervous system

Region of neurone isolation/ recording	Agonist	Antagonist/inhibitor	Receptor	Effect	Desensitization	Reference
Cerebellum	2MeSATP > ADP > ATP > ADO ≫ α,β-meATP > AMP > UTP	GDPβS	P2Y	K^+ channel	—	Ikeuchi and Nishizaki, 1996a
Inferior colliculus	2MeSATP > ADP > ATP > AMP > α,β-meATP	GDPβS	P2Y	K^+ channel	—	Ikeuchi and Nishizaki, 1995b
Superior colliculus	2MeSATP > ADP > ADO > ATP ≫ AMP (UTP, α,β-meATP inactive)	—	P2Y	K^+ channel	—	Ikeuchi *et al.*, 1995b
Dorsal motor nucleus of vagus	ATP (α,β-meATP inactive)	Suramin, RB2 GDPβS-insensitive	P2X	Rapid inward current	No	Nabekura *et al.*, 1995
Hippocampus	ATP	Suramin	—	Slow inward currents[a]	No	Inoue *et al.*, 1992
Hippocampus	2MeSATP > ATP > ADP > α,β-meATP	Suramin	—	Inward currents	—	Balachandran and Bennett, 1996
Hippocampus	ADP ≥ 2MeSATP > ATP > ADO ≫ AMP (UTP, α,β-meATP inactive)	GDPβS	P2Y	K^+ channel, ↑ $[Ca^{2+}]_i$	—	Ikeuchi *et al.*, 1996a,b
Hippocampus	ATP = ADP ≫ AMP > α,β-meATP	↑ $[Ca^{2+}]_i$ via PKC	P2Y	↑ $[Ca^{2+}]_i$	—	Mironov, 1994
Hypoglossal nucleus	ATP	Suramin, PPADS	P2	Excitation of hypoglossal nerve	—	Funk *et al.*, 1997
	ATP inactive	—	—	No inward currents	—	Nabekura *et al.*, 1995
Hypothalamus	ATP, ATPγS > α,β-meATP (ADP inactive)	Suramin	P2X	Rapid ↑ $[Ca^{2+}]_i$	Slow (>100 s)	Chen *et al.*, 1994a
Locus ceruleus	ATP, α,β-meATP	—	P2X	↑ Firing, depolarization	No	Harms *et al.*, 1992
Locus ceruleus	α,β-meATP, 2MeSATP	Suramin, α,β-meATP	P2X	↑ Firing, depolarization	No	Tschöpl *et al.*, 1992
Locus ceruleus	2MeSATP > ATP = ADP > α,β-meATP	—	—	↑ Conductance; depolarization	Slow	Shen and North, 1993
Locus ceruleus	α,β-meATP	Suramin, PPADS	P2X	Rapid depolarization	No	Nieber *et al.*, 1997
		Suramin, PPADS	P2X	Block of synaptic potentials	—	
Medial habenula	ATP, α,β-meATP	Suramin	P2X	Inward currents	Yes	Edwards *et al.*, 1992
		Suramin, α,β-meATP	P2X	Block of synaptic potentials	(>100 ms)	
Medial vestibular nucleus	α,β-meATP	Suramin, PPADS	P2X	↑ Firing	No	Chessell *et al.*, 1997
	ADPβS	PPADS (not suramin)	P2Y	↑ Firing	Slow	
Medulla	2MeSATP > ADP > ATP ≥ α,β-meATP ≥ AMP > UTP	GDPβS	P2Y	K^+ channel	—	Ikeuchi *et al.*, 1995a
Mesencephalic nucleus	ATP inactive	—	—	Ineffective at evoking inward currents	—	Shen and North, 1993
Mesencephalic nucleus	ATP, ATPγS, α,β-meATP	Suramin	P2X	Inward currents	Slow	Khakh *et al.*, 1997
Nucleus tractus solitarius	ATP	—	—	Inward currents	No	Ueno *et al.*, 1992; Nabekura *et al.*, 1995
Parabrachial nucleus	ATP inactive	—	—	No inward currents	—	Shen and North, 1993
Rostral ventrolateral medulla	ATP, α,β-meATP	Suramin	P2X	↑ Firing	No	Sun *et al.*, 1992
					No, Yes[b]	Ralevic *et al.*, 1996
Striatum	2MeSATP > ATP ≥ α,β-meATP	Suramin, RB2, PTX	P2Y	Dopamine release	—	Zhang *et al.*, 1995
Striatum	ATP ≫ 2MeSATP ≥ ADP > ADO > AMP	—	—	K^+ channel	—	Ikeuchi and Nishizaki, 1995a

Substantia nigra	ATP	—	—	Inward currents at 1 in 12 neurones	—	Nabekura *et al.*, 1995
Supraoptic vasopressin neurones	ATP = α,β-meATP	Suramin	P2X	↑ Firing	No[c]	Day *et al.*, 1993
	—	Suramin	P2	Block of excitation to vagus nerve stimulation		
Supraoptic magnocellular neurosecretory cells (hypothalamic)	α,β-meATP > ATP > UTP > 2MeSATP > βγ-meATP	PPADS	P2X	Depolarization, ↑ input conductance	No	Hiruma and Bourque, 1995
Tuberomammillary nucleus	ATP ≥ 2MeSATP ≫ α,β-meATP ≥ ADP	—	P2X	Rapid inward cation current	No	Furukawa *et al.*, 1994
Dorsal horn of spinal cord	ATP	—	P2X	Inward currents	Yes	Jahr and Jessel, 1983
	ATP	—	—	↑ Excitability	—	Fyffe and Perl, 1984
	ATP, ATPγS	Suramin	P2X	Rapid inward currents	Partial	Li and Perl, 1995
Spinal cord neurones	2MeSATP ≥ ATP ≥ ADP ≫ ADP (AMP, UTP, α,β-meATP inactive)	GDPβS	P2Y	K^+ currents	—	Ikeuchi and Nishizaki, 1996b

[a] A rapid inward current is also observed, but is blocked by a non-NMDA receptor antagonist.
[b] Desensitization observed in a subpopulation of α,β-meATP-sensitive neurones.
[c] Reproducible responses to ATP on rapid application at <1 min intervals.
GDPβS, guanosine-5'-*O*-(2-thiodiphosphate); G protein inhibitor; PTX, pertussis toxin; RB2, reactive blue 2.

ganglion. α,β-meATP generally does not bind to P2Y receptors; it is weak or inactive (EC_{50} values 100 μM) at recombinant receptors $P2X_2$ and $P2X_{4-7}$ and at the likely endogenous P2X receptor couterparts (Collo *et al.*, 1996; Evans and Surprenant, 1996). α,β-meATP-sensitive P2X receptors are sensitive to ATP, 2MeSATP, and α,β-meATP with EC_{50} values of approximately 0.5 to 5 μM, whereas α,β-meATP-insensitive P2X receptors are generally less sensitive to ATP and 2MeSATP (EC_{50} values 8 to 50 μM) (Collo *et al.*, 1996; Evans and Surprenant, 1996).

P2X receptors that are sensitive to α,β-meATP can be divided into two groups according to whether they are (rapidly) desensitizing or are non-desensitizing (see also Section IX.D., Desensitization). α,β-MeATP-sensitive desensitizing P2X receptors are cloned $P2X_1$ and $P2X_3$ receptors and their likely endogenous counterparts. α,β-MeATP-sensitive non-desensitizing P2X receptors include some smooth muscle P2X receptors (Knight and Burnstock, 1993; Dobronyi *et al.*, 1997; Relevic *et al.*, 1997), P2X receptors on adult dorsal root ganglion and nodose ganglion, and guinea-pig coeliac neurons as well as heteromeric $P2X_2P2X_3$ receptors (Krishtal *et al.*, 1988a,b; Evans *et al.*, 1992; Li *et al.*, 1993; Khakh *et al.*, 1995a; Lewis *et al.*, 1995; Wright and Li, 1995).

Notably, L-β,γ-meATP is active at P2X but not at P2Y receptors. It can discriminate between α,β-meATP-sensitive P2X receptors on smooth muscle of vas deferens and those on neurons. It is approximately equipotent with α,β-meATP and ATP at vas deferens and at the recombinant $P2X_1$ receptor when ecto-nucleotidase activity is supressed, but ineffective at P2X receptors of rat vagal neurons, rat nodose ganglion neurons, and guinea-pig coeliac neurons (Trezise *et al.*, 1995; Surprenant, 1996).

ATPγS is an agonist at recombinant $P2X_2$ and $P2X_4$ receptors (Brake *et al.*, 1994; Bo *et al.*, 1995). It is a partial agonist at recombinant $P2X_1$ and $P2X_2$ receptors, as well as at endogenous receptors in vas deferens, PC12 cells, and nodose and coeliac ganglia (Surprenant, 1996) with potency generally less than that of ATP.

PPADS, NF023, and NF279 show selectivity as antagonists at P2X *versus* P2Y receptors (see Section VIII.C.).

F. Distribution and Biological Effects

Tissue distributions of the different cloned P2X receptor proteins are detailed in the section on cloned receptors (see Section IX.B.). Most of the receptor proteins have widespread distributions and most tissues express more than one subtype of P2X receptor, which may lead to heteropolymerization. Exceptions are $P2X_3$, which is only expressed in sensory ganglia (Chen *et al.*, 1995a; Lewis *et al.*, 1995), $P2X_1$, which is the principal subtype expressed in smooth muscle (Valera *et al.*, 1994; Collo *et al.*, 1996), and $P2X_4$, which is the only subtype expressed by acinar cells of salivary glands (Buell *et al.*, 1996b). The principal distribution of P2X receptors is on excit-

able tissue such as smooth muscle and nerves, although they have also been cloned from, or have been shown to be expressed by, endocrine tissues ($P2X_4$; Wang *et al.*, 1996), platelets ($P2X_1$-like; MacKenzie *et al.*, 1996), and promyelocyte HL60 cells ($P2X_1$-like; Buell *et al.*, 1996a).

Autoradiography using [^{3}H]-α,β-meATP, which labels $P2X_1$ and $P2X_3$ receptors, has shown high and low affinity binding sites in vascular smooth muscle, urinary bladder, brain, spinal cord, heart, liver, spleen, and cochlea (Bo and Burnstock, 1990, 1993, 1994; Michel and Humphrey, 1993; Balcar *et al.*, 1995; Mockett *et al.*, 1995). The significance of the two binding sites is not clear, and may represent distinct P2X subtypes, although [^{3}H]α,β-meATP binding to nucleotide-binding proteins cannot be excluded. At least two high affinity binding sites for [^{3}H]α,β-meATP were described in a rat aortic endothelial cell line, one of which was suggested to correspond to labeling of 5′-nucleotidase, advising caution in the use of this radioligand (Michel *et al.*, 1995).

1. CNS. P2X receptors are widely distributed in the CNS; excitation and activation of cation channels by ATP and/or α,β-meATP have been described throughout the brain and spinal cord (table 11). However, despite the widespread distribution of P2X receptors, evidence that ATP acts as a fast excitatory transmitter in the brain has so far been convincingly provided only for the medial habenulla (Edwards *et al.*, 1992; Edwards and Gibb, 1993) and locus coeruleus (Nieber *et al.*, 1997). In these regions, synaptic currents are blocked by suramin and by desensitization with α,β-meATP, and are mimicked by ATP and α,β-meATP. Interestingly, the non-desensitizing receptors $P2X_2$, $P2X_4$, and $P2X_6$ are the most abundantly expressed P2X receptors in the brain (Kidd *et al.*, 1995; Collo *et al.*, 1996), which correlates well with the majority of functional studies that show a lack of desensitization of P2X receptors in the CNS (table 11).

Activation of P2X receptors increases the activity of neurons in the rostral ventrolateral medulla and the pre-Bötzinger region, areas within the brainstem that contribute specifically to central regulation of the cardiovascular system and respiratory drive (Sun *et al.*, 1992; Ralevic *et al.*, 1996, 1998). Pronounced effects on blood pressure and respiratory drive observed on microinjection of ATP and α,β-meATP into these regions indicates a potential role for P2X receptors in central modulation of the cardiovascular and respiratory systems (Sun *et al.*, 1992; Ralevic *et al.*, 1996, 1998). Clarification of the physiological significance of these findings awaits identification of the specific pathways and release of endogenous ATP acting as a mediator of these effects.

There are marked regional differences in excitation by ATP of neurons throughout the brain. For instance, in rat brain, responses to ATP are elicited in 100% of neurons in the locus coeruleus, 96% of neurons in the dorsal motor nucleus, and 25% of neurons in the nucleus tractus solitarius, while neurons in the mesencephalic and parabrachial nucleii are insensitive to ATP (Shen and North, 1993; Nabekura *et al.*, 1995). The functional significance of this is not clear. These values correlate poorly with the reported densities of [^{3}H]α,β-meATP binding in rat brain (Bo and Burnstock, 1994), probably because [^{3}H]α,β-meATP binds most strongly to $P2X_1$ and $P2X_3$ receptors and does not reflect adequately the distribution of other P2X subtypes. A strong correlation between the percentage of cells responding to ATP and ACh/nicotine suggests colocalization of P2X and nicotinic ACh receptors (Nabekura *et al.*, 1995).

2. Sensory nerves. Rapid inward currents are mediated by ATP in the dorsal horn of the spinal cord (Li and Perl, 1995; Li *et al.*, 1997b), and there is evidence for P2X receptor-mediated fast synaptic transmission via ATP in a small subset of dorsal horn neurons (Bardoni *et al.*, 1997). Glutamate evoked release after activation of P2X receptors on dorsal root ganglion neurons indicates a role for presynaptic P2X receptors (Gu and MacDermott, 1997). ATP-gated currents have also been shown on many sensory ganglion neurons (Krishtal *et al.*, 1988a,b; Khakh *et al.*, 1995a; Wright and Li, 1995; Robertson *et al.*, 1996; Li *et al.*, 1993, 1997a,b). $P2X_2P2X_3$ heteropolymeric receptors have been suggested to account for non-desensitizing ATP-gated currents in adult sensory ganglia (Lewis *et al.*, 1995). P2X receptors also been shown in peripheral sensory nerve terminals, on capsaicin-sensitive sensory nerve terminals in canine lung (Pelleg and Hurt, 1996) and rat hindpaw (Bland-Ward and Humphrey, 1997), and in rat tooth pulp sensory neurons (Cook *et al.*, 1997), where they may be involved in nociception. Immunohistochemical studies indicate the involvement of $P2X_3$-like receptors in ATP responses in sensory nerves of tooth pulp (Cook *et al.*, 1997). Together, these findings are consistent with the concept that ATP may be involved in the generation of pain signals via P2X receptors

3. PNS. ATP may act via P2X receptors to mediate transmission between neurons, as first shown by suramin-mediated block of synaptic currents between cultured coeliac ganglion cells (Evans *et al.*, 1992; Silinsky *et al.*, 1992). ATP-gated currents also have been shown on many sympathetic (Cloues *et al.*, 1993; Cloues, 1995; Khakh *et al.*, 1995a) and parasympathetic ganglia (Fieber and Adams, 1991; Abe *et al.*, 1995; Sun and Stanley, 1996)

The presynaptic P2 receptors on postganglionic sympathetic neurons may belong to the P2X receptor family. These include P2 receptors on cultured rat sympathetic neurons that mediate NA release (Boehm, 1994; Boehm *et al.*, 1995), P2 receptors in chick cultured sympathetic neurons that facilitate electrically-evoked [^{3}H]NA release (Allgaier *et al.*, 1994a,b, 1995a,b), and P2X ($P2X_2$-like) receptors in pheochromocytoma cells that mediate NA and dopamine release (Inoue *et al.*, 1991; Majid *et al.*, 1992, 1993; Nakazawa and Inoue, 1992; Ikeda *et al.*,

1996). α,β-MeATP acts at presynaptic P2X-like receptors on cholinergic and nonadrenergic axons of guinea-pig ileum to enhance electrically-evoked release of [^{3}H]choline and [^{3}H]NA, respectively (Sperlagh and Vizi, 1991). Activation of cholinergic nerves in guinea-pig ileum via P2X-like receptors has been proposed (Kennedy and Humphrey, 1994). Multiple P2X receptors, predominantly $P2X_2$-like receptors and rapidly desensitizing P2X receptors ($P2X_1$- or $P2X_3$-like), have been described on guinea-pig myenteric neurons (Zhou and Galligan, 1996). In rat isolated vagus nerve, responses to high, but not low, concentrations of α,β-meATP are resistant to antagonism by suramin and reactive blue 2, but are attenuated by iso-PPADS, suggesting heterogeneity of endogenous P2X receptors (Trezise *et al.*, 1994c). An ATP-gated channel sensitive to suramin and insensitive to UTP mediates NA release from a subpopulation of adrenal chromaffin cells (Castro *et al.*, 1995).

4. Smooth muscle. ATP neurotransmission in the PNS identifies a physiological role for P2X receptors on smooth muscle, and as mediators of excitatory junction potentials (EJPs), depolarization, and constriction (Burnstock, 1990; Burnstock and Ralevic, 1996). The postjunctional response of the vas deferens, and most blood vessels to sympathetic nerve stimulation, is a rapid EJP that is blocked by tetrodotoxin, guanethidine, P2 receptor antagonists, and by desensitization of the $P2X_1$-like receptor with α,β-meATP, but is resistant to α-adrenoceptor blockade (Burnstock, 1990; Von Kügelgen and Starke, 1991). Longer periods of stimulation result in summation of the EJPs and the membrane depolarizes allowing the opening of voltage-dependent Ca^{2+} channels, Ca^{2+} entry, and contraction. The $P2X_1$ protein is the predominant subtype expressed in vascular smooth muscle, although $P2X_4$ transcripts have been shown to be expressed in rat aorta and vena cava (Soto *et al.*, 1996a). This correlates well with the rapid desensitization of ATP and α,β-meATP-mediated contractile responses observed in most smooth muscle preparations (Burnstock and Kennedy, 1985; Ralevic and Burnstock, 1988, 1991a,b).

The rabbit saphenous artery provides a classic example of a vessel in which pharmacological manipulations have been used to identify the relative contributions of NA and ATP to sympathetic neurotransmission (Burnstock and Warland, 1987b; Warland and Burnstock, 1987). In this vessel, sympathetic nerve stimulation produces a contractile response of which less than 30% is blocked by the α_1-adrenoceptor antagonist prazosin, whereas the remainder, the purinergic component, is abolished by α,β-meATP (Burnstock and Warland, 1987b) (fig. 10). The sympathetic origin of the purinergic response is confirmed by the fact that reserpine treatment, which depletes sympathetic nerves of their catecholamine content, fails to abolish nerve-mediated contractions despite a greater than 95% reduction in tissue NA content.

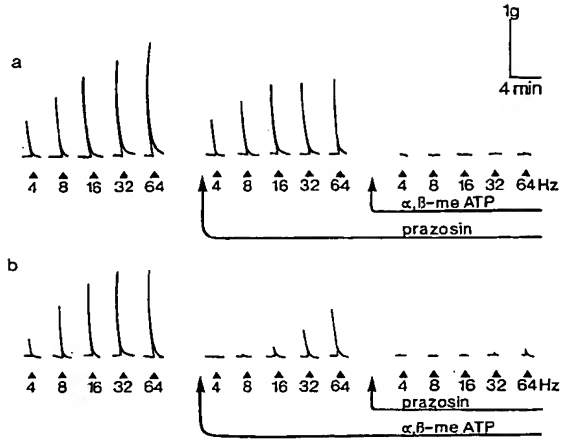

FIG. 10. Contractions produced in the isolated saphenous artery of the rabbit on neurogenic transmural stimulation (0.08–0.1 msec; supramaximal voltage) for 1 sec (a,b) at the frequencies (hz) indicated (▲). Nerve stimulations were repeated in the presence of 10 μM prazosin added before (a) or after (b) desensitization of the P_2-purinoceptor with α,β-methylene ATP (α,β-meATP) as indicated on the figure by the arrowed lines. The horizontal bar signifies 4 min and the vertical bar 1 g. (From Burnstock and Warland, 1987b, *Br J Pharmacol* **90**:111–120; with permission from McMillan Press Limited.)

It can be envisaged that rapid desensitization of the P2X response in smooth muscle may result in attenuation of sympathetic contraction both by effectively eliminating the purinergic component of the response, as well as by removing the potential for synergistic augmentation of the response by postjunctional interactions involving P2X receptors and adrenoceptors (see Ralevic and Burnstock, 1990, 1991a). The physiological significance of rapid desensitization of the smooth muscle P2X receptor is currently unclear, although a role in negative modulation of the sympathetic response during repetitive or prolonged neurogenic stimulation seems to be indicated. The contractile response mediated by P2X receptors in the perfused arterial vasculature of human placental cotyledons is a rare example of a vascular smooth muscle P2X response that does not desensitize (Dobronyi *et al.*, 1997; Ralevic *et al.*, 1997); it may be significant that placental blood vessels are also unique in that they are not innervated.

The expression of more than one functionally-coupled P2X receptor in a single tissue is suggested in the rat vas deferens where three distinct contraction-mediating receptors for ATP were proposed based on differential functional antagonism by PPADS, suramin and reactive blue 2, and different susceptibility to desensitization (Bültmann and Starke, 1994a). Suramin-resistant components of the contractile response to ATP, which may be caused by actions at suramin-insensitive $P2X_4$ and $P2X_6$ receptors, have been described in vas deferens of mouse (Von Kügelgen *et al.*, 1990), rat (Bültmann and Starke, 1994a), and guinea pig (Bailey and Hourani,

1994, 1995), and in frog aorta (Knight and Burnstock, 1996), as well as human urinary bladder (Palea *et al.*, 1995). Where this was examined, the suramin-resistant contractile response to ATP does not appear to be caused by actions at a $P2Y_2$-like receptor, or to ecto-nucleotidase inhibition by suramin (Von Kügelgen *et al.*, 1990; Bailey and Hourani, 1994, 1995; Knight and Burnstock, 1996). A suramin-resistant component of constriction to ATP in cat colon circular muscle also cannot be explained by the ectoATPase activity of suramin (Venkova and Krier, 1993).

Differences in pharmacological profiles have been reported for smooth muscle $P2X_1$-like receptors of urinary bladder, vas deferens, and blood vessels (Abbracchio and Burnstock, 1994; Burnstock *et al.*, 1994). Notably, 2MeSATP and derivatives of ATP are inactive in rabbit saphenous artery but are agonists at $P2X_1$-like receptors in guinea-pig vas deferens and bladder (Burnstock *et al.*, 1994). Non-desensitizing responses of smooth muscle to α,β-meATP have been described in human placental arteries (Dobronyi *et al.*, 1997; Ralevic *et al.*, 1997), and stickleback intestine (Knight and Burnstock, 1993), which is different from the rapidly desensitizing $P2X_1$-like response to α,β-meATP typical of other smooth muscle preparations. It is possible that the non-desensitizing response is mediated by heteromeric P2X receptors with subunits confering both sensitivity to α,β-meATP and resistance to desensitization.

In rat and human urinary bladder, but not in dog bladder, α,β-meATP mediates contraction, suggesting species heterogeneity with respect to expression of P2X receptors in this issue (Palea *et al.*, 1994; Suzuki and Kokubun, 1994). β,γ-MeATP is a potent constrictor of human saphenous vein, but is weak or inactive in human extrarenal veins and arteries (Von Kügelgen *et al.*, 1995a), suggesting that P2X receptor proteins are differentially distributed among vessels.

5. Blood cells. ATP and α,β-meATP activate cation channels in human platelets that have been suggested to be $P2X_1$ receptors (MacKenzie *et al.*, 1996). The currents are mimicked by the spontaneous activation of single channel currents in platelets, suggested to be caused by autocrine activation following release of endogenous ADP and ATP from the platelets. In rat megakaryocytes, ATP and ATPγS activate a rapid (100 ms) nonselective cation channel that rapidly desensitizes (Somasundaram and Mahaut-Smith, 1994), and may also be mediated by a $P2X_1$ receptor. Currents elicited by exogenous ATP or α,β-meATP at $P2X_1$-like receptors in HL60 cells can only be observed when the ongoing desensitization by ATP released from these cells is removed (Buell *et al.*, 1996a), suggesting that $P2X_1$ receptors may be more widely distributed than currently anticipated.

Interactions between P2X and nicotinic ACh receptors, or possibly direct activation by ATP of ACh receptors (possibly by actions on different subunits), have been described in PC12 cells (Nakazawa *et al.*, 1990; Nakazawa, 1994), cultured *Xenopus* myotomal muscle cells (Igusa, 1988), membranes of rat superior cervical ganglion (SCG) cells (Nakazawa and Inoue, 1993; Nakazawa, 1994), and postjunctional ACh receptors in rat cultured flexor digitorum brevis muscle fibers (Mozrzymas and Ruzzier, 1992). ATP-induced [^{3}H]NA release from chick sympathetic neurons is blocked by nicotinic receptor antagonists (Allgaier *et al.*, 1995b). However, ATP does not act at nicotinic receptors in guinea-pig coeliac ganglion (Evans *et al.*, 1992), rat intracardiac neurons (Fieber and Adams, 1991), or, controversially, rat SCG neurons (Cloues *et al.*, 1993; Boehm, 1994).

X. $P2X_7$ and Endogenous $P2X_7$-Like (or P_{2Z}) Receptors

The $P2X_7$ receptor cloned from rat macrophages and brain by Surprenant *et al.* in 1996 is the cytolytic "P_{2Z} receptor" previously described in mast cells, macrophages, fibroblasts, lymphocytes, erythrocytes, and erythroleukemia cells. In line with the main aim of this review, "$P2X_7$-like receptor" is used for the endogenous receptor counterpart of the $P2X_7$ receptor in preference to "P_{2Z} receptor". A unique feature of cloned $P2X_7$ and endogenous $P2X_7$-like receptors is that, whereas under physiological conditions these function like other P2X receptors in that they are selectively permeable to small cations only, in the continued presence of ATP and when divalent cation levels are low, the cation channel can convert to a pore, permeable to small molecules as well as ions.

A. Structure

The $P2X_7$ receptor and its endogenous counterpart is structurally similar to other P2X receptors (see Section IX A), except for the fact that it has a significantly longer intracellular C-terminal (240 amino acids) than other P2X receptors, of which at least the last 177 amino acids are crucial for the induction of the non-selective pore (Surprenant *et al.*, 1996).

B. Cloned $P2X_7$ Receptors

The $P2X_7$ receptor was first cloned from rat brain and macrophages (Surprenant *et al.*, 1996). The recombinant receptor has an agonist potency order for eliciting inward currents of 3′-O-(4-benzoyl)benzoyl ATP (BzATP) $\gg$ ATP $\gg$ 2MeSATP $>$ ATPγS $>$ ADP (Surprenant *et al.*, 1996) (table 9). The human homolog has been cloned and shows a lower sensitivity to agonists (Rassendren *et al.*, 1997). In low divalent cation solution, agonists induce sustained currents and the channel becomes permeable to molecules of up to 900 daltons, although in normal solution selectivity for small cations is observed (Surprenant *et al.*, 1996). As with other P2X receptors, this receptor is inhibited by divalent cations (Rassendren *et al.*, 1997; Virginio *et al.*, 1997).

C. Signal Transduction Mechanisms

Brief activation of the recombinant $P2X_7$ receptor and its endogenous counterpart causes rapid membrane depolarization and cation influx and is a reversible process. However, sustained activation causes an increase in permeability by allowing bidirectional transport of a variety of ions including Na^+, K^+, and Ca^{2+} and small molecules with a molecular weight of less than or equal to 900 daltons, except in lymphocytes where the limit is 200–300 daltons. This effect is associated with cytotoxicity. Permeabilization involves the cytoplasmic C terminus of the protein because it does not occur with a truncated $P2X_7$ receptor lacking the last 177 residues, although cation function of the receptor is retained. The different upper size limit of the pore for $P2X_7$-like receptors in different cells may represent isoforms of the receptor or different conductance states.

In murine and human macrophages (el-Moatassim and Dubyak, 1992, 1993; Humphreys and Dubyak, 1996) and human leukaemic lymphocytes (Gargett *et al.*, 1996; Gargett and Wiley, 1997), activation of $P2X_7$-like receptors causes activation of phospholipase D, although the mechanism is unknown. In lymphocytes this has been suggested to be coupled to the influx of bivalent cations (Gargett *et al.*, 1996), whereas in murine macrophages it is suggested to occur distinct from $P2X_7$-like pore formation (el-Moatassim and Dubyak, 1993). In murine macrophages BzATP-induced activation of phospholipase D is not mimicked by Ca^{2+}-mobilizing agonists or by activators of protein kinase C (el-Moatassim and Dubyak, 1992), and in a human monocyte cell line it is blocked by calcium-calmodulin kinase II inhibition (Humphreys and Dubyak 1996).

Activation of the $P2X_7$-like receptor of human macrophages triggers the release of the inflammatory cytokine IL-1β, which may provide a clue to the physiological and/or pathophysiological role of this receptor (Griffiths *et al.*, 1995; Ferrari *et al.*, 1997).

D. Desensitization

Currents evoked at recombinant $P2X_7$ and endogenous $P2X_7$-like receptors do not readily desensitize. However, species differences in the time for which the current flows caused by brief application of agonist have been described. Currents elicited by BzATP at the recombinant rat $P2X_7$ receptor decline slowly, particularly in low divalent cation solution, leading to sustained currents (10–20 min) even by very brief agonist application (1–3s) (Surprenant *et al.*, 1996). By contrast, currents evoked at the human $P2X_7$ receptor decline to baseline within 10–20 sec of discontinuing agonist application (Rassendren *et al.*, 1997).

E. Agonists

The recombinant $P2X_7$ receptors and its endogenous counterpart have high selectivity for ATP, with most other purine compounds having little or no activity. The active ligand is suggested to be the tetrabasic acid ATP^{4-} (Cockcroft and Gomperts, 1980), which is present as approximately 1% of the relatively high concentration (100 μM) of ATP that is required to activate this receptor. Thus, reducing the extracellular cation concentration increases agonist potency. Increasing the concentration of Mg^{2+} rapidly closes the cation channel, although it is not clear to what extent this is due to the formation of the inactive $MgATP^{2-}$ complex, caused by direct block of the ion channel, or caused by a decrease in affinity caused by allosteric modulation of the receptor (Virginio *et al.*, 1997). By contrast with other P2X receptors, the $P2X_7$-like receptor is inhibited by Cu^{2+} and Zn^{2+} (Virginio *et al.*, 1997). P^1,P^4-diadenosine tetraphosphate (Ap_4A) can activate the $P2X_7$-like receptor of mast cells, possibly because of its quadruple negative charge (Tatham *et al.*, 1988).

BzATP is currently the most potent agonist at the endogenous $P2X_7$-like receptor; it is 10 to 100 times more potent than ATP in activating $P2X_7$-like receptors in a number of cells (Gonzalez *et al.*, 1989a; Erb *et al.*, 1990; el-Moatassim and Dubyak, 1992; Soltoff *et al.*, 1992; McMillian *et al.*, 1993; Nuttle *et al.*, 1993), although it is only twice as potent as ATP in eliciting cytolysis of hepatocytes (Zoetewij *et al.*, 1996). Species differences between human and murine macrophage $P2X_7$-like receptors have been suggested, based on different sensitivities to permeabilization by ATP, BzATP, and ATPγS (Hickman *et al.*, 1994).

F. Antagonists

KN-62 (1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine) has been described as a potent antagonist at the $P2X_7$-like receptor of human lymphocytes with an IC_{50} of approximately 12 nM (Gargett and Wiley, 1997).

2′,3′-Dialdehyde ATP (oxidized ATP) is an antagonist at the $P2X_7$-like receptor, but is irreversible and requires prolonged exposure of cells to high concentrations of inhibitor (Murgia *et al.*, 1993; Wiley *et al.*, 1994; Falzoni *et al.*, 1995; Humphreys and Dubyak, 1996; Zoetewij *et al.*, 1996; Surprenant *et al.*, 1996).

G. Distribution and Biological Effects

$P2X_7$ mRNA and protein are distributed in bone marrow cells, including granulocytes, monocytes/macrophages and B lymphocytes, and in macrophages in brain, as shown by evidence from functional studies on these cell types (Collo *et al.*, 1997).

Functional studies have shown that $P2X_7$-like receptor distribution is generally limited to cells of hemopoietic origin including mast cells (Cockcroft and Gomperts, 1980; Tatham *et al.*, 1988; Tatham and Lindau, 1990), macrophages (Steinberg *et al.*, 1987; Greenberg *et al.*, 1988; el-Moatassim and Dubyak, 1992, 1993; Murgia *et al.*, 1992, 1993; Hickman *et al.*, 1994; Falzoni *et al.*,

1995), the human monocyte cell line THP-1 (Humphreys and Dubyak, 1996), fibroblasts (Weisman *et al.*, 1989; Erb *et al.*, 1990; Pizzo *et al.*, 1992), erythrocytes (Parker and Snow, 1972), erythroleukaemia cells (Chahwala and Cantley, 1984), and lymphocytes (Wiley *et al.*, 1994; Gargett *et al.*, 1996; Jamieson *et al.*, 1996; Markwardt *et al.*, 1997). $P2X_7$-like receptors are also present on hepatocytes (Zoetewij *et al.*, 1996) and parotid and salivary gland acinar cells (Sasaki and Gallacher, 1990; McMillian *et al.*, 1993; Soltoff *et al.*, 1992, 1993).

Although several roles for the $P2X_7$ receptor have been proposed, its physiological significance is largely unknown. The increased permeability caused by activation of the $P2X_7$-like receptor results in large ion fluxes and leakage of small metabolites. On prolonged stimulation it may cause cell swelling, vacuolization, and cell death by necrosis or apoptosis (Dubyak and el-Moatassim, 1993). The biological significance of this cytotoxic effect of ATP is not clear, but may have a role in the elimination of unwanted cells during physiological or pathological cell and tissue turnover. There is increasing evidence to support suggestions that the $P2X_7$ receptor is involved in signaling between macrophages or other cells involved in the immune response and target cells (Steinberg and Di Virgilio, 1991; Dubyak and el-Moatassim, 1993); the $P2X_7$-like receptor is involved in fusion of macrophages to form multinucleated giant cells that die shortly after fusion, a process that is inhibited by oxidized ATP (Chiozzi *et al.*, 1997). Furthermore, ATP causes the release of the inflammatory cytokine IL-1β via the $P2Y_7$-like receptor of human macrophages (Griffiths *et al.*, 1995; Ferrari *et al.*, 1997).

Loss of the adhesion molecule L-selectin from leukocytes after activation of $P2X_7$-like receptors implicates a role for these receptors in modulation of leukocyte binding to endothelial cells and migration through the vascular wall (Jamieson *et al.*, 1996; Wiley *et al.*, 1996).

XI. P2Y Receptors

P2Y receptors are purine and pyrimidine nucleotide receptors that are coupled to G proteins. Currently this includes the cloned mammalian receptors $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$, and the $P2Y_{ADP}$ (or P_{2T}) receptor (that has not yet been cloned), and endogenous uridine nucleotide-specific receptors (that show some pharmacological similarities with cloned $P2Y_4$ and $P2Y_6$ receptors) (tables 10 and 12). The chick p2y3 receptor may be the homolog of the human $P2Y_6$ receptor (hence lower case lettering). Putative $P2Y_5$, $P2Y_7$, $P2Y_9$, and $P2Y_{10}$ receptors are not included in the definitive P2Y receptor family after convincing evidence that these are not P2Y receptors. A receptor claimed as $P2Y_{Ap4A}$ (or P_{2D}) has not yet been cloned, but may belong to the P2Y receptor family. A P2Y receptor has been cloned from *Xenopus* neural plate (Bogdanov *et al.*, 1997).

Receptors for pyrimidines that are activated specifically by uridine nucleotides, but not by adenine nucleosides or nucleotides, were first proposed by Seifert and Schultz in 1989. This proposal has been confirmed by the cloning of two uridine nucleotide-specific receptors, $P2Y_4$ (human) and $P2Y_6$, showing preference for UTP and UDP, respectively (Communi *et al.*, 1996b, c) (but see Section XV). Subsequent to Seifert and Schultz's proposal, but before the cloning of $P2Y_4$ and $P2Y_6$ receptors, some confusion in the literature was caused by the identification of "P_{2U}-purinoceptors", activated equipotently by UTP and ATP (O'Connor *et al.*, 1991), because P_{2U} receptors were often loosely termed "pyrimidinoceptors" and separate identity of these and receptors activated preferentially by UTP or UDP (but weakly or not at all by ATP) was often indistinct. The cloning of the $P2Y_2$ receptor and its characterization as a receptor activated by ATP, as well as UTP, helped to reinforce the concept that this receptor is distinct from receptors that are activated selectively by pyrimidines.

A. Structure

P2Y receptors are 308 to 377 amino acid proteins with a mass of 41 to 53 kDa after glycosylation. The seven transmembrane domain tertiary structure of P2Y receptors is common to that of other G protein-coupled receptors, general features of which have been described for adenosine P1 receptors (see Section.II.B.). A model of the P2Y receptor, based on the primary sequence of the $P2Y_1$ receptor and using the structural homolog rhodopsin as a G protein-coupled receptor template, has identified positively charged amino acid residues in transmembrane regions 3, 6, and 7 that may be involved in ligand binding by electrostatic interactions with the phosphates of ATP (Van Rhee *et al.*, 1995). Several of these amino acids are conserved in other G protein-coupled receptors. Site-directed mutagenesis of the $P2Y_2$ receptor to convert positively charged amino acids in transmembrane regions 6 and 7 to neutral amino acids causes a 100- to 850-fold decrease in the potency of ATP and UTP, which suggests a role for these amino acids in binding purines and pyrimidines (Erb *et al.*, 1995). By contrast, the most critical residues for ATP binding at the human $P2Y_1$ receptor are in transmembrane regions 3 and 7 on the exofacial side of the receptor (Jiang *et al.*, 1997).

Most P2Y receptors act via G protein coupling to activate PLC leading to the formation of IP_3 and mobilization of intracellular Ca^{2+}. Coupling to adenylate cyclase by some P2Y receptors has also been described. The response time of P2Y receptors is longer than that of the rapid responses mediated by P2X receptors because it involves second-messenger systems and/or ionic conductances mediated by G protein coupling. Signaling pathways for the P2Y receptor subtypes are considered in detail in the sections for each of these receptors.

XII. $P2Y_1$ and Endogenous $P2Y_1$-Like Receptors

The $P2Y_1$ receptor, and its endogenous counterpart termed $P2Y_1$-like, is a receptor for the endogenous ligands ADP, ATP, and certain diadenosine polyphosphates; it is not activated by UDP and UTP. It seems to be more sensitive to adenine nucleotide diphosphates than to triphosphates. Sensitivity to ATP seems to be variable; many $P2Y_1$ and $P2Y_1$-like receptors are relatively insensitive to ATP (ATP may act as a partial agonist), but are strongly activated by ADP (see Heterogeneity of $P2Y_1$-like receptors, Section XII.F.). Characteristically, among all other P2Y subtypes, the $P2Y_1$ receptor and its endogenous counterpart are strongly activated by 2MeSATP, ADP, ADPβS, and adenosine-5′-O-(2-fluoro)-diphosphate (ADPβF) (table 10b). In the present review, evidence for G protein coupling, and evidence that 2MeSATP and ADP or ADPβS or ADPβF are full and potent agonists, is taken as provisional evidence for an endogenous $P2Y_1$-like receptor, although this remains to be confirmed with the development and use of selective agonists and antagonists.

A. Cloned $P2Y_1$ Receptors

The first cloned $P2Y_1$ receptor was from chick brain (Webb *et al.*, 1993b) (table 12). The recombinant receptor is activated by agonists with a potency order of 2MeSATP ≥ ATP ≫ ADP, although α,β-meATP, β,γ-meATP, and UTP are inactive (Webb *et al.*, 1993b). Responses to ATP and 2MeSATP are antagonized by suramin and reactive blue 2. Activation of the recombinant $P2Y_1$ receptor mediates IP_3 formation and an increase in intracellular Ca^{2+}, but no change in cAMP levels (Simon *et al.*, 1995). Homologs of the chick brain $P2Y_1$ receptor have been cloned from a variety of species (table 12). Notably, the relative potency of ATP and ADP differs widely between recombinant $P2Y_1$ and endogenous $P2Y_1$-like receptors. Although it is possible that for recombinant receptors this is because of differences in assay conditions, the unequivocal insensitivity to ATP of some endogenous $P2Y_1$-like receptors (Dixon *et al.*, 1995; Ralevic and Burnstock, 1996a; Webb *et al.*, 1996b) suggests that this is likely to be due to inherent differences in receptor structure.

B. Signal Transduction Mechanisms

The main signal transduction pathway of recombinant $P2Y_1$ and endogenous $P2Y_1$-like receptors is activation of PLC. From studies of the $P2Y_1$-like receptor in turkey erythrocytes, the G protein has been identified as a G_q protein, G_{11}, and is insensitive to pertussis and cholera

TABLE 12
Cloned P2Y receptors

Receptor	Number of amino acids	cDNA library source	Agonist activity	References
$P2Y_1$	362	Human brain	2MeSATP > ATP ≫ UTP	Schachter *et al.*, 1996
		Human prostate and ovary	2MeSATP > ATP = ADP	Janssens *et al.*, 1996
		Human placenta	—	Léon *et al.*, 1995, 1997
		Human HEL cells	—	Ayyanathan *et al.*, 1996
		Bovine endothelium	2MeSATP = ADP > ATP ≫ UTP	Henderson *et al.*, 1995
		Rat insulinoma cells	2MeSATP > 2Cl-ATP > ATP (α,β-meATP inactive)	Tokuyama *et al.*, 1995
		Rat ileal myocytes	2MeSATP = 2ClATP > ADP > ATP (UTP inactive)	Pacaud *et al.*, 1996
		Mouse insulinoma cells	—	Tokuyama *et al.*, 1995
		Turkey brain	2MeSATP > ADP > ATP (UTP inactive)	Filtz *et al.*, 1994
		Chick brain	2MeSATP > ATP > ADP (UTP inactive)	Webb *et al.*, 1993b
$P2Y_2$	373	Human CF/T43 epithelial cells	ATP = UTP ≫ 2MeSATP	Parr *et al.*, 1995
		Human bone	—	Bowler *et al.*, 1995
		Rat microvascular coronary EC	—	Gödecke *et al.*, 1996
		Rat alveolar type II cells	ATP = UTP	Rice *et al.*, 1995
		Rat pituitary	ATP = UTP > ADP = UDP > GTP	Chen *et al.*, 1996b
		Wistar Kyoto rat[a]	—	Seye *et al.*, 1996
		Mouse NG108-15 neuroblastoma cells	ATP = UTP > ATPγS ≫ 2MeSATP	Lustig *et al.*, 1993
p2y3[b]	328	Chick brain	UDP > UTP > ADP > 2MeSATP > ATP	Webb *et al.*, 1995, 1996a
$P2Y_4$	352	Human placenta	UTP > ATP = ADP[c]	Communi *et al.*, 1996b
		Human placenta	—	Stam *et al.*, 1996
		Human chromosome X	UTP > UDP (ATP inactive)	Nguyen *et al.*, 1996
		Rat heart	ATP = UTP = ADP = ITP = ATPγS = 2MeSATP = Ap_4A > UDP	Bogdanov *et al.*, 1998
$P2Y_6$	379	Human placenta and spleen	UDP > UTP > ADP > 2MeSATP ≫ ATP	Communi *et al.*, 1996b
		Rat aortic smooth muscle	UTP > ADP = 2MeSATP > ATP	Chang *et al.*, 1995
		Activated T-cells	—	Southey *et al.*, 1996
$P2Y_{11}$	371	Human placenta	ATP > 2MeSATP >>> ADP (UTP, UDP inactive)	Communi *et al.*, 1997

[a] Tissue not specified.
[b] p2y3 may be the chick homologue of the mammalian $P2Y_6$ receptor.
[c] The reported activity of UDP at the $P2Y_4$ receptor has been shown to be caused by UTP present as a contaminant.

toxin, which activates PLCβ isoenzymes via its α subunit (Waldo *et al.*, 1991a, 1991b; Maurice *et al.*, 1993). Insensitivity or partial sensitivity to pertussis toxin is characteristic of most endogenous $P2Y_1$-like receptors coupled to PLC, indicating the involvement of $G_{q/11}$ proteins. In contrast, $P2Y_1$-like receptors coupled to inhibition of adenylate cyclase are typically blocked by pertussis toxin, indicating an involvement of G_i proteins (Boyer *et al.*, 1995; Berti-Mattera *et al.*, 1996; Webb *et al.*, 1996c).

IP_3 formation and Ca^{2+} mobilization can stimulate a variety of signaling pathways including PKC, PLA_2, Ca^{2+}-dependent K^+ channels, NOS and subsequent endothelium-derived relaxing factor (EDRF) formation, and can generate endothelium-derived hyperpolarizing factor (EDHF). The main physiological target of DAG is stimulation of PKC, which in turn may stimulate phosphatidyl choline-specific PLC, PLD, the MAPK pathway, and Ca^{2+} influx via voltage-operated Ca^{2+} channels. Generation of PKC (with no detectable elevations in IP_3 or cytosolic Ca^{2+}) and subsequent rapid tyrosine phosphorylation of MAPK seems to be the pathway by which $P2Y_1$-like (and $P2Y_2$-like) receptors on endothelial cells mediate prostacyclin production (Bowden *et al.*, 1995; Patel *et al.*, 1996). This pathway is involved in cell metabolism, secretion, gene expression, and growth. $P2Y_1$-like receptor activation of a phosphatidyl choline-specific PLC, and of PLD, has been reported (Martin and Michaelis, 1989; Pirotton *et al.*, 1990; Purkiss and Boarder, 1992), although activation may occur downstream of PKC.

A second signaling pathway of endogenous $P2Y_1$-like receptors may be inhibition of adenylate cyclase. This has been described for $P2Y_1$-like receptors in a clonal population of rat brain capillary endothelial cells (B10 cells) (Webb *et al.*, 1996c). The two pathways are expressed independently, that is, $P2Y_1$-like activation of PLC does not coincide with $P2Y_1$-like inhibition of adenylate cyclase. It is not yet clear whether this involves differential G protein-coupling or is caused by heterogeneity of $P2Y_1$-like receptors (Webb *et al.*, 1996c). P2Y receptor-mediated adenylate cyclase inhibition was originally described for $P2Y_1$-like receptors in rat C6 glioma cells and the clonal cell line C6–2B (Pianet *et al.*, 1989; Valeins *et al.*, 1992; Lin and Chuang, 1993; Boyer *et al.*, 1993, 1994, 1995). However, the decrease in cAMP in C6 cells is not blocked by selective antagonists of the $P2Y_1$ receptor,which suggests that these receptors are distinct from $P2Y_1$ receptors coupled to activation of PLC (Boyer *et al.*, 1996). $P2Y_1$-like receptor-mediated inhibition of adenylate cyclase activity has also been described in Schwann cells (Berti-Mattera *et al.*, 1996). Inhibition of adenylate cyclase is pertussis toxin-sensitive, indicating an involvement of G_i proteins, but it is unclear whether activation is mediated by α, β, or γ subunits (Boyer *et al.*, 1995; Harden *et al.*, 1995; Webb *et al.*, 1996c).

$P2Y_1$-like receptors may mediate membrane-delimited G protein regulation of ion channels, that is, lack the involvement of cytosolic second-messenger systems. Although membrane-delimited regulation is frequently assumed to imply a direct physical interaction between the active G protein subunit and the ion channel, some ion channels may be regulated by lipid-soluble second-messengers such as arachidonic acid and metabolites (Wickman and Clapham, 1995). In rat cerebellar neurons, the opening of an outwardly rectifying, pertussis toxin-insensitive GDPβS-sensitive K^+ current by 2MeSATP > ADP > ATP activation of a $P2Y_1$-like receptor was suggested via coupling of the β,γ subunits of the G protein to a K^+ channel (Ikeuchi and Nishizaki, 1996a). The single channel currents induced by 2MeSATP were without latency, suggesting that the channel was activated only by plasma membrane factors without the involvement of intracellular components (Ikeuchi and Nishizaki, 1996a). An ADP-sensitive K^+ channel in inferior colliculus (Ikeuchi and Nishizaki, 1995b) and medullar (Ikeuchi *et al.*, 1995a) neurons was also suggested to be activated by direct action of the $\beta\gamma$ subunits of the G protein. In contrast, 2MeSATP and ATP activation of a K^+ channel in striatal neurons seems to be mediated via PKC (Ikeuchi and Nishizaki, 1995a).

In some cells, $P2Y_1$-like receptors are colocalized with $P2Y_2$-like receptors. The biological significance of this is not clear, particularly where ATP is a common agonist, but makes more sense where the $P2Y_1$-like receptor is selective for ADP, and ATP acts only at the $P2Y_2$-like receptor (as has shown to be the case for coexisting $P2Y_1$- and $P2Y_2$-like receptors on some endothelial cells). The receptors have similar signaling pathways, although the $P2Y_1$-like receptor seems to be more sensitive than the $P2Y_2$-like receptor to manipulations of PKC activity. This is likely to be related to the important role of PKC as a negative feedback regulator of PLC activity to allow finely tuned regulation of this signaling pathway. Thus, stimulation of PKC with 12-O-tetradecanoyl-β-phorbol 13-acetate (TPA) causes a greater inhibition of $P2Y_1$- than of $P2Y_2$-like receptor mediated responses in rat osteoblastic cells (Gallinaro *et al.*, 1995). The IP_3 response of the endothelial $P2Y_1$-like receptor is attenuated by stimulation of PKC with phorbol 12-myristate 13-acetate and enhanced by PKC inhibition with Ro 31–8220, but the $P2Y_2$-like response is less affected or is unaffected (Purkiss *et al.*, 1994; Communi *et al.*, 1995; Chen *et al.*, 1996a). Discrimination between the signaling pathways of $P2Y_1$- and $P2Y_2$-like receptors, and the ways in which these may be differentially modulated, might provide some clues about the biological significance of their colocalization.

C. Desensitization

In general, $P2Y_1$ and $P2Y_1$-like receptors do not readily desensitize. When this does occur, as with other G protein-coupled receptors, desensitization may in-

volve receptor phosphorylation by protein kinases and uncoupling from the associated G protein. Studies of the $P2Y_1$-like receptor in turkey erythrocyte membranes showed that desensitization ($t_{1/2}$ 15 min) is heterologous, involves multiple mechanisms, and does not involve PKC or intracellular Ca^{2+} (Galas and Harden, 1995). In cultured bovine aortic endothelial cells, preexposure to 2MeSATP or UTP causes homologous partial desensitization of IP_3 formation by $P2Y_1$- and $P2Y_2$-like receptors, respectively, and heterologous partial desensitization of the 2MeSATP response by UTP (Wilkinson *et al.*, 1994). $P2Y_1$-like receptor desensitization has also been observed in rat colon muscularis mucosae (Hourani *et al.*, 1993) and rabbit mesenteric arterial smooth muscle (Ziganshin *et al.*, 1994b).

D. Agonists

The $P2Y_1$ and $P2Y_1$-like receptor is generally more sensitive to adenine nucleotide diphosphates than to triphosphates. ADPβS, ADPβF, and 3′-deoxyATPαS (dATPαS) are potent agonists at $P2Y_1$ receptors. 2MeSATP is a potent and selective agonist at the $P2Y_1$ and $P2Y_1$-like receptor *versus* other cloned P2Y receptors (but see $P2Y_{11}$ receptor, Section XVII.), but is also a potent agonist at most P2X receptors. α,β-meATP, β,γ-meATP, and UTP are inactive and thus are useful as negative evidence in the characterization of this receptor. Certain of the diadenosine polyphosphates (particularly those with a phosphate chain of three phosphates or less) may be natural, albeit non-selective, agonists at $P2Y_1$-like receptors (Ralevic *et al.*, 1995a; Pintor *et al.*, 1996). The potency of ATP differs widely among endogenous $P2Y_1$-like receptors, and the lack of effect of ATP at some endogenous $P2Y_1$-like receptors is unequivocal (Dixon *et al.*, 1995; Ralevic and Burnstock, 1996a; Webb *et al.*, 1996b). This would tend to rule out the possibility that this heterogeneity is caused by contamination of solutions of ADP and ATP caused by purine interconversion and metabolism. However, molecular evidence does not support a subdivision of the $P2Y_1$ receptor, and heterogeneity of ADP/ATP relative potencies is also apparent for recombinant $P2Y_1$ receptors (table 12).

The charge carried by the molecule may influence agonist potency; it has been suggested that ATP uncomplexed with divalent cations, ATP^{4-}, is the preferred agonist of the $P2Y_1$-like receptor expressed on bovine aortic endothelial cells (Motte *et al.*, 1993b). In the guinea-pig taenia coli, the order of potency for relaxation at the $P2Y_1$-like receptor by non-hydrolysable analogs of β,γ-meATP reflects the order of electronegativity, with the more acidic analogs being more potent: AMP-PCF_2P > AMP-CCl_2P > β,γ-meATP (Cusack *et al.*, 1987).

2-Thioether derivatives of adenine nucleotides, including 2-hexylthio ATP and 2-cyclohexylthio ATP, are potent agonists at $P2Y_1$-like receptors coupled to adenylate cyclase (EC_{50} values 28 and 58 pM respectively), but are significantly less potent at PLC-coupled $P2Y_1$ receptors (Boyer *et al.*, 1995). N^6-Methyl ATP is selective for $P2Y_1$-like receptors in the taenia coli versus vascular $P2Y_1$-like receptors (Fischer *et al.*, 1993; Burnstock *et al.*, 1994).

E. Antagonists

Adenosine 3′,5′- and 2′,5′-bisphosphates act as competitive antagonists at the $P2Y_1$ receptor coupled to PLC; adenosine-3′-phosphate-5′-phosphosulfate (A3P5PS) and adenosine-3′-phosphate-5′-phosphate (A3P5P) block responses at the recombinant $P2Y_1$ receptor with pK_B values of 6.5 and 5.7, respectively (Boyer *et al.*, 1996). These compounds are inactive at the adenylate cyclase-coupled $P2Y_1$-like receptor of C6 glioma cells and at recombinant $P2Y_2$, $P2Y_4$, or $P2Y_6$ receptors (Boyer *et al.*, 1996). Interestingly, A3P5PS and A3P5P are partial agonists at the turkey but not the human recombinant $P2Y_1$ receptor. N^6-methyl modification of 2′-deoxyadenosine 3′5′-bisphosphate, to produce the compound MRS 2179, enhanced antagonist potency (IC_{50} value 330 nM) by 17-fold and eliminated the partial agonist properties observed with the lead compound, resulting in the most potent $P2Y_1$ receptor antagonist reported to date (Camaioni *et al.*, 1998).

F. Heterogeneity of $P2Y_1$ and Endogenous $P2Y_1$-Like Receptors

Although endogenous $P2Y_1$-like receptors couple to different signal transduction pathways and there may be profound differences in their ligand binding profiles, molecular evidence does not support the subdivision of this receptor. It seems most likely that this heterogeneity may arise from small differences in structure. Sequence homology of only 84% between turkey and human $P2Y_1$ receptors may explain why A3P5PS and A3P5P are partial agonists at the turkey $P2Y_1$ receptor but not its human homolog (Boyer *et al.*, 1996). These receptors were expressed in the same cell type and assayed under the same conditions.

Heterogeneity in ligand binding at $P2Y_1$ receptors includes both agonist and antagonist binding profiles. Recombinant $P2Y_1$ receptors cloned from different species and tissues show different relative potencies to ATP and ADP (table 12), as do their endogenous counterparts. Although the true potency of ATP at endogenous $P2Y_1$-like receptors is difficult to assess because of actions at coexisting receptors and rapid breakdown by ecto-nucleotidases, ADP-specific $P2Y_1$-like receptors that are activated potently by ADP and 2MeSATP, but weakly or not at all by ATP, have been described in a number of isolated cells and tissues, including rat hepatocytes (Keppens and deWulf, 1991; Keppens *et al.*, 1992; Dixon *et al.*, 1995), endothelium of rat mesenteric arteries (Ralevic and Burnstock, 1996a,) and rat brain capillary endothelial cells (Feolde *et al.*, 1995; Webb *et al.*, 1996c). The P2 receptor antagonist PPADS has been shown to block vasodilatation mediated by ADP and

2MeSATP (at a $P2Y_1$-like receptor) but not to ATP and UTP (at a $P2Y_2$-like receptor), which implies that at least in rat mesenteric arteries, ATP does not act at $P2Y_1$-like receptors, although it does act at $P2Y_2$-like receptors (Ralevic and Burnstock, 1996a). This has important implications for the agonist selectivity of $P2Y_1$ receptors in other tissues.

ADP-specific $P2Y_1$-like receptors may account for some of the ambiguities in the literature concerning classification of P2Y receptors. Thus, ADP-activated P2Y receptors identified as "P_{2T}" ($P2Y_{ADP}$) receptors in osteoblasts (Sistare *et al.*, 1994, 1995) are likely to be ADP-specific $P2Y_1$ receptors because 2MeSATP and ADP are equipotent agonists (Reimer and Dixon, 1992; Sistare *et al.*, 1994, 1995; Dixon *et al.*, 1997b). A "P_{2T}" receptor coexisting with the $P2Y_2$ receptor in porcine ovarian granulosa cells may also be an ADP-specific $P2Y_1$ receptor (Kamada *et al.*, 1994).

PPADS is able to discriminate between some $P2Y_1$ receptors; it generally blocks recombinant $P2Y_1$ receptors and endogenous $P2Y_1$-like receptors coupled to PLC (Boyer *et al.*, 1994; Brown *et al.*, 1995; Charlton *et al.*, 1996a; Schachter *et al.*, 1996) but has no effect at $P2Y_1$-like receptors coupled to inhibition of adenylate cyclase (Boyer *et al.*, 1994; Webb *et al.*, 1996c). On the other hand, PPADS is ineffective at rabbit aortic endothelial $P2Y_1$-like receptors, where PLC coupling might be expected (Ziganshin *et al.*, 1994b). Block of $P2Y_1$-like receptors with different pA_2 values also implies receptor heterogeneity: pA_2 values 5.1 and 5.3 in rat duodenum and guinea-pig taenia coli, respectively, (Windscheif *et al.*, 1995a); pA_2 values 6.0 in rat mesenteric arterial endothelium (Ralevic and Burnstock, 1996a) and at recombinant turkey brain (Charlton *et al.*, 1996a) $P2Y_1$ receptors. PPADS is ineffective as an antagonist at rabbit mesenteric arterial smooth muscle $P2Y_1$-like receptors (Ziganshin *et al.*, 1994b).

Different sensitivities to ATP and analogs of ATP have been shown for $P2Y_1$-like receptors in guinea-pig taenia coli, and in vascular endothelium and smooth muscle (Fischer *et al.*, 1993; Burnstock *et al.*, 1994; Abbracchio and Burnstock, 1994). Among other differences, N^6-methylATP is a selective agonist at guinea-pig taenia coli $P2Y_1$-like receptors, but is inactive at vascular $P2Y_1$-like receptors (Fischer *et al.*, 1993; Burnstock *et al.*, 1994). Relaxation by α,β-meATP of the guinea-pig taenia coli seems to be via a P2Y receptor of undetermined subtype as this response is not blocked by the P2X-selective antagonist Evans blue (Bültmann *et al.*, 1996). 2-Thioether derivatives of adenine nucleotides are potent agonists at adenylyl cyclase-linked $P2Y_1$-like receptors in C6 rat glioma cells, but not at PLC-linked $P2Y_1$-like receptors of turkey erythroctyes (Boyer *et al.*, 1995). Interestingly, ATP seems to be a partial agonist at adenylate cyclase-coupled P2Y receptors. At the endothelial $P2Y_1$-like receptor, P^1,P^3-diadenosine triphosphate (Ap_3A) is the most potent ligand and P^1,P^5-diadenosine pentaphosphate (Ap_5A) is inactive (Ralevic *et al.*, 1995a).

G. Distribution and Biological Effects

$P2Y_1$ and $P2Y_1$-like receptors are widely distributed having been described in heart, vascular, connective, immune, and neural tissues. The transcript for chick brain $P2Y_1$ mRNA is distributed in brain, spinal cord, gastrointestinal tract, spleen, and skeletal muscle, but not in heart, liver, stomach, lung, or kidney (Webb *et al.*, 1993b). In the rat, $P2Y_1$ receptor mRNA is expressed at variable levels in many tissues including heart, brain, spleen, lung, liver, skeletal muscle, and kidney, but is not detected in testis (Tokuyama *et al.*, 1995). Within the brain, $P2Y_1$ mRNA has a widespread but specific distribution, being particularly rich in various nuclei of the telencephalon, diencephalon, and mesencephalon as well as in the external granule, Purkinje, and internal granule cells of the cerebellum (Webb *et al.*, 1994).

Receptors with the pharmacological profile of a $P2Y_1$ receptor have been identified in functional studies in a wide variety of cells including rat astrocytes (Pearce *et al.*, 1989; Pearce and Langley, 1994), frog glial cells (Robitaille, 1995), avian erythrocytes (Berrie *et al.*, 1989; Boyer *et al.*, 1989), rat osteoblasts (Reimer and Dixon, 1992; Gallinaro *et al.*, 1995), pancreatic β cells (Petit *et al.*, 1988), rat mast cells (Osipchuk and Cahalan, 1992), rat alveolar type II cells (Rice and Singleton, 1987), human T-leukemia cells (Biffen and Alexander, 1994), rat cochlear lateral wall (Ogawa and Schacht, 1995), and rat cochlear lateral wall epithelial cells (Ikeda *et al.*, 1995). The physiological significance of these receptors is still largely undetermined. Diverse $P2Y_1$-like receptor-mediated metabolic effects include insulin secretion from pancreatic β-cells (Bertrand *et al.*, 1987; Hillaire-Buys *et al.*, 1991, 1993, 1994), renin secretion in renal cortical slices (Churchill and Ellis, 1993a, 1993b), gluconeogenesis in renal cortical tubules (Cha *et al.*, 1995), and glycogenolysis in rat hepatocytes (Keppens and De Wulf, 1991).

The distribution of $P2Y_1$-like receptors on vascular endothelium and smooth muscle cells implies a role in the regulation of vascular tone. In most blood vessels, $P2Y_1$-like receptors are present on the endothelium and mediate vasodilatation by Ca^{2+}-dependent activation of endothelial NOS and generation of EDRF and by generation of EDHF. Endothelial prostacyclin production is also stimulated by the $P2Y_1$-like receptor, but this seems to play a minimal role in vasodilatation, at least under physiological conditions. The fact that ATP and ADP are released locally from endothelial cells during shear stress and hypoxia and from platelets during aggregation, identifies a possible role for endothelial $P2Y_1$-like receptors in modulation of vascular tone under normal conditions and during thrombosis. $P2Y_1$-like receptors on pulmonary artery endothelium may be involved in stimulation of leukocyte adhesion (Dawicki *et al.*, 1995).

$P2Y_1$-like receptors are present on the smooth muscle of a number of blood vessels and, like their endothelial counterparts, mediate vasodilatation (Kennedy and Burnstock, 1985; Mathieson and Burnstock, 1985; Burnstock and Warland, 1987a; Liu *et al.*, 1989; Brizzolara and Burnstock, 1991; Keefe *et al.*, 1992; Corr and Burnstock, 1994; Qasabian *et al.*, 1997; Simonsen *et al.*, 1997). $P2Y_1$-like receptors (and $P2Y_2$-like receptors) are expressed by human coronary artery smooth muscle cells in culture (Strøbæk *et al.*, 1996). The mechanism underlying relaxation by smooth muscle $P2Y_1$-like receptors is not known but may involve activation of K^+ channels. In rabbit mesenteric arteries and skeletal muscle-resistance arteries, glibenclamide partially blocks smooth muscle hyperpolarization and relaxation to ADP, indicating a role for K_{ATP} channels (Brayden, 1991). The smooth muscle $P2Y_1$-like receptor of rabbit pulmonary artery mediates relaxation independently of mobilization of intracellular Ca^{2+} (in contrast with that mediated by coexisting $P2Y_2$-like receptors) implying lack of involvement of the PLC pathway (Qasabian *et al.*, 1997). The biological significance of $P2Y_1$-like receptors expressed by the smooth muscle of rabbit portal vein (Brizzolara *et al.*, 1993) (fig. 11), guinea-pig pulmonary artery (Liu *et al.*, 1992), and lamb small coronary arteries (Simonsen *et al.*, 1997) may be in mediation of the neurogenic, purinergic (non-adrenergic non-cholinergic) relaxation shown in these vessels. It is possible that vascular smooth muscle $P2Y_1$-like receptors mediate relaxation to ATP released as a neurotransmitter from sensory-motor nerves. A $P2Y_1$-like receptor on cultured aortic smooth muscle cells has been reported to mediate the mitogenic effect of ATP via activation of PKC, and then Raf-1 and MAPK (Yu *et al.*, 1996); it has also been reported to cause induction of immediate early genes (Malam-Souley *et al.*, 1996), which indicates a role in vascular smooth muscle proliferation.

Interestingly, autocatalytic release of ATP (ATP-mediated release of ATP) has been described in guinea-pig cardiac endothelial cells, which may involve $P2Y_1$-like receptors (Yang *et al.*, 1994). A $P2Y_1$-like receptor on rat basophilic leukocyte cells is suggested to amplify intracellular Ca^{2+} signaling and secretory responses to antigen stimulation, and to propagate the response to neighboring cells partly by the release of additional stores of ATP from secretory granules (Osipchuk and Cahalan, 1992).

Activation of the $P2Y_1$-like receptor expressed on platelets leads to platelet shape change, aggregation, and intracellular calcium rise, with no effect on adenylate cyclase (Daniel *et al.*, 1998; Hechler *et al.*, 1998; Jin *et al.*, 1998). This effect is blocked by the selective $P2Y_1$ receptor antagonists A2P5P and A3P5P. The $P2Y_1$ receptor seems to be crucial for triggering the ADP-induced shape change, whereas aggregation is mediated by cooperative effects with platelet $P2Y_{ADP}$ (or P_{2T}) receptor-mediated inhibition of adenylate cyclase (Daniel *et al.*, 1998; Hechler *et al.*, 1998; Jin *et al.*, 1998).

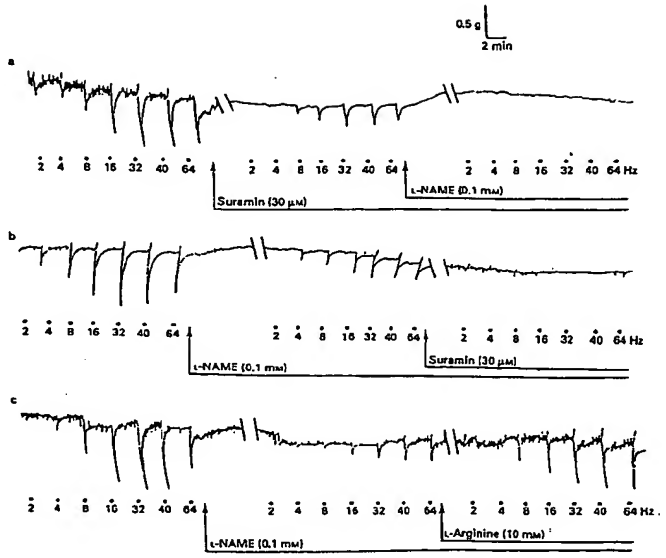

FIG. 11. Relaxations of the rabbit portal vein to neurogenic transmural stimulation for 10 sec (2 to 64 Hz, 0.7 ms, 100 V) at 5 min intervals. Guanethidine (3.4 μM) and atropine (0.114 μM) were present throughout to block adrenergic and cholinergic neurotransmission respectively. Tone was induced with ergotamine (8.6 μM). Panel (a) shows that preincubation with suramin (30 μM) for 20 min reduced the nerve-mediated relaxations compared with controls and that suramin-resistant neurogenic relaxations were abolished 20 min after the addition of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 0.1 mM). Panel (b) shows that neurogenic relaxations remaining after 20 min pretreatment of the tissue with L-NAME (0.1 mM) were abolished 20 min after the addition of suramin (30 μM). In (c), the effect of adding L-NAME (0.1 mM) to the tissue is shown; there was an additional rise in tone and inhibition of the response to nerve stimulation after a 20 min incubation period. The subsequent treatment of tissues with L-arginine (10 mM) for 20 min reversed this effect. Each of the traces in (a), (b), and (c) is representative of similar results in six separate experiments. (From Brizzolara *et al.*, 1993, *Br J Pharmacol* **109**:606–608; with permission from McMillan Press Limited).

$P2Y_1$ receptor mRNA is selectively expressed by large diameter sensory neurons and when expressed in oocytes was shown to be mechano-sensitive and to exhibit inward currents (Nakamura and Strittmatter, 1996). A functional correlate may be ATP-triggered Ca^{2+} release from IP_3-sensitive Ca^{2+} stores in large DGR neurons; $[Ca^{2+}]_i$ transients were not elicited by small neurons (Svichar *et al.*, 1997).

ATP inhibits the light-evoked release of ACh from rabbit retinal cholinergic neurons in a DPCPX-insensitive manner, although the receptor subtype is not clear (Neal and Cunningham, 1994). A $P2Y_1$-like receptor may mediate inhibition by ATP and 2MeSATP (but not α,β-meATP) of excitatory postsynaptic potentials in guinea-pig submucosal neurons, and although it is suggested that it is a P3-like receptor, it is not activated by adenosine (Barajas-López *et al.*, 1995).

$P2Y_1$-like receptors mediate the opening of K^+ channels in rat cultured cerebellar neurons, striatal neurons, superior and inferior colliculus neurons, medullar neu-

rons, hippocampal neurons, and spinal neurons (Ikeuchi *et al.*, 1995a,b; 1996a,b; Ikeuchi and Nishizaki, 1995b; 1996a,b). The transduction mechanism seems to be a pertussis toxin-insensitive G protein which directly opens the potassium channels via its $\beta\gamma$ subunit. Adenosine seems to be an agonist at $P2Y_1$-like receptors in hippocampal neurons (Ikeuchi *et al.*, 1996a) and neurons of the superior colliculus (Ikeuchi *et al.*, 1995b), raising the possibility that these are P1 or P3 receptors. A $P2Y_1$-like receptor mediates dopamine release in rat striatum (Zhang *et al.*, 1995). An increase in the firing rate of rat medial vestibular nucleus neurons by ADPβS has been attributed to activation of P2Y receptors (Chessell *et al.*, 1997).

XIII. $P2Y_2$ and Endogenous $P2Y_2$-Like Receptors

The $P2Y_2$ receptor (and its endogenous counterpart, formerly called the P_{2U} receptor) is activated by ATP and UTP with approximately equal potency and is insensitive or is only weakly activated by ADP and other nucleoside diphosphates, 2MeSATP and α,β-meATP (table 10b). In this review, endogenous receptors exhibiting this pharmacological profile have provisionally been termed $P2Y_2$-like (but see Section XV.).

A. Cloned $P2Y_2$ Receptors

The first cloned $P2Y_2$ receptor was from mouse NG108–15 neuroblastoma cells (Lustig *et al.*, 1993). Species homologs have been cloned from rat, cat, and human (table 12).

B. Signal Transduction Mechanisms

Cloned $P2Y_2$ and endogenous $P2Y_2$-like receptors couple via both $G_{i/o}$ and $G_{q/11}$ proteins to mediate phospholipid breakdown and phosphoinositides as well as Ca^{2+} mobilization via PLCβ, an effect which may accordingly be pertussis toxin-sensitive, -partially sensitive, or -insensitive (see Dubyak and el-Moatassim, 1993). $P2Y_2$-like receptor coupling to G_i proteins involves the $\beta\gamma$ G_i protein subunits, which stimulate phospholipase C-β_2. IP_3 formation, Ca^{2+} mobilization, and a variety of signaling pathways including PKC, PLA_2, Ca^{2+}-dependent K^+ channels, and EDRF and EDHF formation. The specific downstream involvement of a given signaling pathway seems to be partially dependent on the cell type in which the $P2Y_2$-like receptor is expressed.

Activation of PLD and stimulation of phosphatidylcholine breakdown by $P2Y_2$-like receptors has been reported (Purkiss and Boarder, 1992; Pfeilschifter and Merriweather, 1993; Balboa *et al.*, 1994; Gerwins and Fredholm, 1995a,b). The mechanism of activation of PLD is unclear but may involve the combined actions of PKC, Ca^{2+}, and G proteins, as suggested for $P2Y_2$-mediated pertussis toxin-insensitive activation of PLD in DDT_1 MF2 cells (Gerwins and Fredholm, 1995b). As with the $P2Y_1$-like receptor, protein tyrosine phosphorylation and MAPK activation seems to be the major route for $P2Y_2$-like receptor-mediated prostacyclin production in endothelial cells (Bowden *et al.*, 1995; Patel *et al.*, 1996). This occurs subsequent to activation of PKC and does not involve IP_3 or cytosolic Ca^{2+} (Patel *et al.*, 1996). Stress-activated protein kinases, independent of PKC activation, have been shown to be activated by ATP and UTP in rat renal mesangial cells (Huwiler *et al.*, 1997).

Secondary to activation of PLC and mobilization of Ca^{2+}, the $P2Y_2$-like receptor mediates the opening of Ca^{2+}-sensitive Cl^- channels in airway epithelia (Clarke and Boucher, 1992; Stutts *et al.*, 1992), intrahepatic biliary epithelial cell lines (Wolkoff *et al.*, 1995), and avian exocrine salt gland cells (Martin and Shuttleworth, 1995), which drives fluid secretion. Activation of $P2Y_2$-like receptors stimulates cation and K^+ currents via Ca^{2+}-dependent signaling mechanisms in HTC cells from a rat liver tumor cell line (Fitz and Sostman, 1994). UTP and ATP mediate depolarization of supraoptic neurosecretory cells in rat hypothalamus by the opening of a non-selective cation channel (Hiruma and Bourque, 1995).

A $P2Y_2$-like receptor has been shown to mediate inhibition of adenylate cyclase in some cells, although as shown in C6–2B rat glioma cells, this may occur secondary to an increase in cytosolic free Ca^{2+} (Munshi *et al.*, 1993). Inhibition of cAMP accumulation by UTP and ATP at a $P2Y_2$-like receptor in NCB-20 cells is accompanied by an elevation in intracellular Ca^{2+} (Garritsen *et al.*, 1992). A pertussis toxin-sensitive G protein mediates $P2Y_2$-like inhibition of cAMP accumulation in cultured renal mesangial cells (Schulze-Lohoff *et al.*, 1995). In the renal epithelial cell line, MDCK-D1 cells UTP and ATP mediate an increase in cAMP that is blocked by indomethacin identifying a cyclooxygenase-dependent mechanism; this suggests the involvement of PGE_2 (Post *et al.*, 1996). An increase in cGMP levels mediated by $P2Y_2$-like receptors in mouse neuroblastoma × rat glioma hybrid cells occurs secondary to mobilization of intracellular Ca^{2+} (Reiser, 1995).

Inhibition of N-type calcium currents by $P2Y_2$-like receptors expressed in sympathetic neurons has been reported (Filippov *et al.*, 1997).

$P2Y_2$-like receptors are colocalized with $P2Y_1$-like receptors on many cells and have a common signaling pathway in PLC. $P2Y_2$-like responses are less sensitive to manipulations of the PKC pathway (Purkiss *et al.*, 1994; Communi *et al.*, 1995; Gallinaro *et al.*, 1995; Chen *et al.*, 1996a) (see also Section XII.B., on $P2Y_1$ and $P2Y_1$-like receptor signal transduction mechanisms).

C. Desensitization

$P2Y_2$ and endogenous $P2Y_2$-like receptors do not readily desensitize. However, tachyphylaxis of a $P2Y_2$-like response has been reported in UMR-106 rat osteoblasts (Sistare *et al.*, 1994), human term placental (trophoblastic) cells (Petit and Belisle, 1995), rat cultured

pituitary cells (gonadotropes) (Chen *et al.*, 1994b, 1995b), C6–2B rat glioma cells (Munshi *et al.*, 1993), and in cultured endothelial cells (Motte *et al.*, 1993a; Wilkinson *et al.*, 1994; Nobles *et al.*, 1995). Maximum desensitization of the $P2Y_2$ receptor in mouse epithelial cells was observed at 5 to 10 min after UTP exposure, and full receptor responsiveness recovered at the same time after removal of agonist (Garrard *et al.*, 1998). The mechanism of desensitization is not well understood, but as with many G protein-coupled receptors may involve phosphorylation of the intracellular regions of the receptor. The C terminal may be important because progressively larger truncations of this region of the $P2Y_2$ receptor decreased the rate and magnitude of desensitization (Garrad *et al.*, 1998).

Plasticity of expression of the $P2Y_2$ receptor during in vitro differentiation and inflammatory activation of HL-60 human promyelocytic leukocytes has been described (Martin *et al.*, 1997a). When HL-60 cells differentiate into neutrophils, $P2Y_2$ receptor mRNA levels and receptor function are largely preserved. In contrast, differentiation of HL-60 cells into monocytes/macrophages is associated with a complete loss of $P2Y_2$ receptor-mediated function and a 10-fold reduction of $P2Y_2$ mRNA levels; this suggests receptor down-regulation (Martin *et al.*, 1997a). It was suggested that down-regulation of the $P2Y_2$-like receptor might be related to inflammatory activation rather than differentiation.

D. Up-Regulation

$P2Y_2$-like receptor activity and $P2Y_2$ receptor mRNA levels were increased in rat submandibular gland after ligation of the main excretory duct but not in the contralateral nonligated gland, indicating that changes in expression of the $P2Y_2$ receptor may occur during pathological conditions (Turner *et al.*, 1997).

E. Agonists and Antagonists

UTP and ATP are natural ligands at $P2Y_2$ and $P2Y_2$-like receptors, and are approximately equipotent. 2MeSATP and α,β-meATP are weak or inactive, which provides useful negative evidence in the characterization of this receptor. UTPγS is equipotent with UTP and ATP at recombinant $P2Y_2$ and endogenous $P2Y_2$-like receptors, but has the advantage of being resistant to hydrolysis (Lazarowski *et al.*, 1996). ATPγS has been shown to be an agonist at recombinant $P2Y_2$ receptors, but is less potent than UTP and ATP (Lustig *et al.*, 1993; Lazarowski *et al.*, 1995). Ap_4A is a potent agonist at recombinant $P2Y_2$ receptors with a potency greater than ATPγS and is within the same range as UTP and ATP, raising the possibility that it is an endogenous regulator of these receptors (Lazarowski *et al.*, 1995).

It has been suggested that endogenous $P2Y_2$-like receptors are preferentially activated by the fully ionized forms of ATP and UTP, ATP^{4-}, and UTP^{4-} in bovine aortic endothelial cells (Lustig *et al.*, 1992; Motte *et al.*, 1993b), human neutrophils (Walker *et al.*, 1991), a cultured neuroblastoma-glioma hybrid cell line (NG108–15 cells) (Lin *et al.*, 1993), rat lactotrophs (Carew *et al.*, 1994), mouse pineal gland tumor cells (Suh *et al.*, 1997), and MDCK cells (Yang *et al.*, 1997). The UTP and ATP responses were shown to correlate with the concentration of the fully ionized form of these agonists and not with the concentration of their cation complexes or other ionized forms. Although both UTP and ATP are rapidly degraded and augmentation of responses in Mg^{2+}-free medium by ecto-nucleotidases must be considered, this seems not to be involved because potentiation of responses was also observed for the stable agonist ATPγS (Yang *et al.*, 1997). Direct effects of cations on the receptor are also possible.

There are no selective antagonists at $P2Y_2$ and $P2Y_2$-like receptors. Suramin and PPADS are nonselective antagonists at subpopulations of $P2Y_2$-like receptors (see Section XIII.F., Heterogeneity of $P2Y_2$ and Endogenous $P2Y_2$-Like Receptors).

F. Heterogeneity of $P2Y_2$ and Endogenous $P2Y_2$-Like Receptors

Endogenous $P2Y_2$-like receptors show two phenotypes of response with respect to antagonism by suramin and PPADS. However, there is no molecular evidence to support a subdivision of $P2Y_2$ receptors. The differences in sensitivities to antagonists do not correspond to species differences or to the apparent division according to differences in G protein coupling. Suramin-insensitive $P2Y_2$-like receptors are those on bovine aortic endothelial cells (Wilkinson *et al.*, 1994), rat duodenum muscularis mucosae (Johnson *et al.*, 1996), rabbit aortic endothelium (Chinellato *et al.*, 1994), and rat mesenteric arterial endothelium (Ziyal, 1997). PPADS-insensitivity is also reported for $P2Y_2$-like receptors on rat mesenteric arterial endothelium (Ralevic and Burnstock, 1996a), as well as for $P2Y_2$-like receptors on rat renal artery smooth muscle (Eltze and Ullrich, 1996) and bovine aortic endothelial cells (Brown *et al.*, 1995).

Suramin-sensitive endogenous $P2Y_2$-like receptors include those on mouse C2C12 myotubes (Henning *et al.*, 1992, 1993), rat pituitary gonadotrophs (Chen *et al.*, 1994b), mouse cortical thick ascending limb segments (Paulais *et al.*, 1995), rat lactotrophs (Carew *et al.*, 1994), hamster mesenteric endothelium (Ziyal, 1997), rat PC12 cells (Murrin and Boarder, 1992), DDT MF-2 cells (Hoiting *et al.*, 1990; Sipma *et al.*, 1994), rat astrocytes (Ho *et al.*, 1995), early embryonic chick neural retina (Sugioka *et al.*, 1996; but also see Section XVII. on Endogenous Uridine Nucleotide-Specific Receptors), rat brain endothelial cells (Nobles *et al.*, 1995), rabbit pulmonary artery endothelium and cultured smooth muscle cells (Qasabian *et al.*, 1997), bovine pulmonary artery endothelium (Chen *et al.*, 1996c), mouse mammary tumor epithelial cells (Enomoto *et al.*, 1994), and mouse neuroblastoma and rat glioma hybrid cells (Reiser, 1995). PPADS is also an inhibitor of $P2Y_2$-like receptors

in mouse neuroblastoma and rat glioma hybrid cells (Reiser, 1995), as well as of $P2Y_2$-like receptors in rat astrocytes (Ho *et al.*, 1995).

G. Distribution and Biological Effects

$P2Y_2$ and endogenous $P2Y_2$-like receptors are widely distributed, but relatively little is known about their physiological significance. Particularly intriguing is the functional significance of a receptor that can be activated equally by purines and pyrimidines; to establish the physiological relevance of this it is important to know more about whether there are different sources or differential release of UTP and ATP. Some of these questions may be answered in the not too distant future as a result of the recent development of a radiometric assay based on the nucleotide specificity of UDP-glucose pyrophosphohydrolase, which is capable of detecting nanomolar concentrations of UTP (Lazarowski *et al.*, 1997a). UTP has been shown to be released from endothelial cells by increased flow (Saiag *et al.*, 1995) and is released from epithelial and astrocytoma cells by perturbation of the bathing medium (mechanical stimulation) (Enomoto *et al.*, 1994; Lazarowski *et al.*, 1997a). ATP is also released from these cells under these conditions, although whether its release is independent of that of UTP is unclear. UTP is stored in platelets (Goetz *et al.*, 1971), which may be significant in modulation of vascular contractility during platelet aggregation in pathophysiological conditions.

Northern blot analysis revealed distribution of $P2Y_2$ receptor mRNA in spleen, testes, kidney, liver, lung, heart, and brain (Lustig *et al.*, 1993; Parr *et al.*, 1995). Alveolar type II cell $P2Y_2$ receptor mRNA is expressed in rat heart, kidney, lung, spleen, and testis, but not in brain or liver (Rice *et al.*, 1995). The $P2Y_2$ receptor cloned from human osteoclastoma is expressed in osteoclastoma, bone, and osteoblasts (Bowler *et al.*, 1995). $P2Y_2$ receptor mRNA has been localized in primary cultures of rat aortic smooth muscle cells (Chang *et al.*, 1995) and in cardiac myocytes and fibroblasts (Webb *et al.*, 1996d).

As shown in functional studies, receptors exhibiting the pharmacological properties of the $P2Y_2$ receptor are present in a wide variety of cells and tissues including astrocytes, different types of blood cells, chromaffin cells, endothelial cells, epithelial cells, fibroblasts, glial cells, hepatocytes, keratinocytes, myocytes, osteoblasts, pancreatic β-cells, pheochromocytoma PC12 cells, pituitary cells, thyrocytes, and tumor cells (table 13).

In the vasculature, $P2Y_2$-like receptors are generally present on the endothelium where they stimulate the synthesis and release of prostacyclin and NO, leading to vasodilatation (Ralevic and Burnstock, 1991a, 1991b; 1996a, 1996b). Smooth muscle contraction mediated equipotently by UTP and ATP may indicate $P2Y_2$-like receptors, although the G protein coupling of these receptors remains to be confirmed. These receptors have been described in rat pulmonary vasculature (Rubino and Burnstock, 1996), rat renal vasculature (Eltze and Ullrich, 1996), bovine middle cerebral artery (Miyagi *et al.*, 1996a), and rat duodenum (Johnson *et al.*, 1996). Interestingly, Ca^{2+}-mobilizing $P2Y_2$-like receptors described on cultured smooth muscle cells of rabbit pulmonary artery are not coupled to a functional response (Qasabian *et al.*, 1997). A clue to their role may lie in the demonstration that $P2Y_2$-like receptors mediate an increase in expression of immediate-early and delayed-early cell cycle-dependent genes in cultured aortic smooth muscle cells, in contrast with the induction only of immediate-early genes by 2MeSATP in the same cells (Malam-Souley *et al.*, 1996).

Enhanced leukocyte adherence to cultured pulmonary artery endothelial cells by $P2Y_2$-like receptors has been shown (Dawicki *et al.*, 1995). $P2Y_2$ receptors on neutrophils stimulate degranulation, potentiate N-formyl-methionyl-leucyl-phenylalanine (FMLP)-induced superoxide formation, and induce aggregation (Kuroki *et al.*, 1989; Seifert *et al.*, 1989a,b; Walker *et al.*, 1991). $P2Y_2$-like receptors on HL-60 cells mediate activation of NADPH oxidase and superoxide generation and mediate potentiation of FMLP-induced superoxide formation (Seifert *et al.*, 1989a), while those on neutrophils and HL-60 cells induce chemotaxis and actin polymerization (Verghese *et al.*, 1996). $P2Y_2$-like receptors on gonadotrophs mediate the release of luteinizing hormone (Chen *et al.*, 1995b). $P2Y_2$-like receptors are Cl^- secretagogues in human nasal mucosa, probably via activation of Ca^{2+}-dependent Cl^- channels (Mason *et al.*, 1991; Stutts *et al.*, 1992); this is an effect which has been explored for its potential in the pharmacological control of cystic fibrosis, a disease characterized by a failure to secrete Cl^- ions into the airway lumen leading to dehydration of airway secretions.

Coupling of $P2Y_2$-like receptors to catecholamine secretion in PC12 cells is controversial, having been reported by some researchers (Majid *et al.*, 1993; Koizumi *et al.*, 1995b), but not by others (Barry and Cheek, 1994; Nikodijevic *et al.*, 1994; de Souza *et al.*, 1995). It is intriguing that while there is no good evidence for UTP release as a neurotransmitter, it is able to modulate the release of other substance from nerves.

It has been shown recently (Bogdanov *et al.*, 1998) that, unlike the human $P2Y_4$ receptor (see Section XV.), which is selective for UTP, the rat $P2Y_4$ homolog is equisensitive to ATP and UTP; that is, in agonist profile it is identical with rat $P2Y_2$. Therefore, it seems likely that the endogenous receptor called $P2Y_2$-like in this section may be a $P2Y_2$ or a $P2Y_4$ receptor, at least where rat tissue is concerned. However, since there is a differential sensitivity to widely used antagonists, it should be possible to distinguish which receptor is operating in a particular tissue. In view of this new data, it is now clear that the former P_{2U} receptor cannot be equated with a single P2Y subtype.

XIV. p2y3 Receptor

This receptor has been cloned from chick brain and has nucleotide selectivity with a potency order of UDP > UTP > ADP > 2MeSATP > ATP (Webb *et al.*, 1995, 1996a). The designation p2y3 reflects the current reservations expressed by the IUPHAR nomenclature committee about its inclusion as a distinct subtype within the P2Y receptor family because no mammalian homolog has yet been identified. It has been suggested that this may be the chick homolog of the mammalian $P2Y_6$ receptor, with which it has 62% sequence homology, although this has not yet been confirmed. This receptor is activated by UDP, and to a lesser extent UTP and ADP, and couples to PLC. Its expression is rather restricted, being detected in spleen, spinal cord, kidney, and lung.

XV. $P2Y_4$ Receptor

This uridine nucleotide-specific receptor has been cloned from human placenta (Communi *et al.*, 1996c), human chromosome X (Nguyen *et al.*, 1996), and rat heart (Bogdanov *et al.*, 1998). The human $P2Y_4$ receptor is highly selective for UTP over ATP and is not activated by nucleoside diphosphates. ATP can act as an antagonist and partial agonist. The human $P2Y_4$ receptor seems to couple to two distinct G proteins: a G_i protein at the early stage and a $G_{q/11}$ protein at a later stage of signaling to activate PLC and IP_3 formation (Communi *et al.*, 1996a). The IP_3 response declines within minutes of stimulation of the receptor and is not readily reproducible, indicating desensitization (Robaye *et al.*, 1997). The human $P2Y_4$ receptor is not blocked by suramin, but has been reported to be both blocked by PPADS (IC_{50} approximately 15 μM) (Communi *et al.*, 1996a) and to be relatively insensitive to block by PPADS (used at 30 μM) (Charlton *et al.*, 1996b). $P2Y_4$ has a restricted distribution; it is expressed almost exclusively in placenta with low levels of expression in lung, and absent in most other tissues. A $P2Y_4$ receptor (initially termed P2P) has been described in rat pancreas (Stam *et al.*, 1996). $P2Y_4$ mRNA (and $P2Y_2$ mRNA, as well as barely detectable levels of $P2Y_6$ mRNA) has been detected in vascular smooth muscle (Erlinge *et al.*, 1998).

The recent cloning of a rat $P2Y_4$ receptor has shown that the recombinant receptor is activated equipotently by ATP and UTP (ADP, ATPγS, 2MeSATP, and Ap_4A are also equipotent, but are partial agonists) (Bogdanov *et al.*, 1998). Clearly, with respect to ATP and UTP sensitivity, this is identical with the profile described for the $P2Y_2$ receptor. Important implications arising from this are that some $P2Y_2$-like responses may be mediated by a $P2Y_4$ receptor, at least in rat tissues, and that the P_{2U} receptor cannot be equated with a single P_{2Y} subtype.

XVI. $P2Y_6$ Receptor

This uridine nucleotide-specific receptor has been cloned from rat aortic smooth muscle (Chang *et al.*, 1995) and human placenta and spleen (Communi *et al.*, 1996b). The receptor is activated most potently by UDP but weakly or not at all by UTP, ATP, ADP, or 2MeSATP (Communi *et al.*, 1996b; Nicholas *et al.*, 1996). Other diphosphonucleotides are full agonists at the receptor but have lower affinities. The response is pertussis toxin insensitive, indicating the involvement of $G_{q/11}$ proteins in stimulation of PLC and in the formation of IP_3. Interestingly, the IP_3 response of the human cloned $P2Y_6$ receptor decays only slowly after stimulation, remaining above baseline for more than an hour after stimulation; this is a response that is fully reproducible without the need for a long recovery period (Robaye *et al.*, 1997).

$P2Y_6$ mRNA is found abundantly in various rat tissues including placenta, thymus, lung, stomach, intestine, spleen, mesentery, heart, and aorta (Chang *et al.*, 1995; Communi *et al.*, 1996b). $P2Y_6$, along with $P2Y_1$ and $P2Y_2$, but not $P2Y_4$ mRNA, has been detected in adult rat cardiac myocytes (Webb *et al.*, 1996d). It has been suggested that the $P2Y_6$ receptor accounts for uridine nucleotide-specific responses in C6–2B cells (Nicholas *et al.*, 1996). A receptor activated by UDP in human nasal epithelial cells that is distinct from the $P2Y_2$ receptor may be an endogenous $P2Y_6$ receptor (Lazarowski *et al.*, 1997b). The receptor promotes [^{3}H]inositol phosphate accumulation and an increase in $[Ca^{2+}]_i$ and Cl^- secretion, is present on the mucosal but not on the serosal surface, and desensitizes more readily than responses to UTP (Lazarowski *et al.*, 1997b). Interestingly, a uridine nucleotide-specific receptor responding to UDP in Caco-2 human intestinal epithelial cells seems to be located on the apical but not on the basolateral membrane (Inoue *et al.*, 1997). The more widespread distribution of the $P2Y_6$ receptor, compared with the $P2Y_4$ receptor, suggests that this receptor is more likely to account for endogenous uridine nucleotide-specific responses.

XVII. $P2Y_{11}$ Receptor

The $P2Y_{11}$ receptor was cloned from human placenta (Communi *et al.*, 1997). The receptor has 33% amino acid identity with the $P2Y_1$ receptor, its closest homolog, and 28% homology with the $P2Y_2$ receptor. The receptor couples to the stimulation of both the phosphoinositide and the adenylyl cyclase pathways; in this respect, it is unique among the P2Y family. Interestingly, this receptor seems to be the only P2Y receptor selective for ATP because it is stimulated by agonists with a rank order of potency of ATP > 2MeSATP >>> ADP, with UTP and UDP inactive (Communi *et al.*, 1997). Northern blot analysis detected mRNA corresponding to the $P2Y_{11}$ receptor in spleen and HL-60 cells (Communi *et al.*, 1997).

TABLE 13
Functional distribution of P2Y receptors

	$P2Y_1$-like[a]	$P2Y_2$-like[b]	$P2Y_{ADP}$[c]	Uridine nucleotide-specific	References
Alveolar type II cells	Yes	Yes	—	—	Rice and Singleton, 1987; Rice *et al.*, 1995
Astrocytes	Yes	Yes	—	—	Pearce and Langley, 1994; Salter and Hicks, 1994; Ho *et al.*, 1995; Chen and Chen, 1996
Blood cells					
Erythrocytes	Yes	—	—	—	Boyer *et al.*, 1989, 1994
Erythroleukemic (human HEL megakaryocytes)	Yes	Yes	Yes	—	Shi *et al.*, 1995
Leukemic basophils (rat mast cells)	Yes	—	—	—	Osipchuk and Cahalan, 1992; Qian and McCloskey, 1993
T-leukemia cells	Yes[d,e]	—	Yes[e]	—	Biffen and Alexander, 1994
Macrophages	—	Yes	—	Yes	Greenberg *et al.*, 1988; Nuttle *et al.*, 1993; Lin and Lee, 1996
Megakaryocytes	—	—	Yes	—	Vittet *et al.*, 1992; Uneyama *et al.*, 1994
Monocytes (murine J774)	Yes	—	—	—	Fan and McCloskey, 1994
Myelomonocytic leukemic (M1)	Yes	—	—	—	Yamaguchi *et al.*, 1994
Neutrophils	—	Yes	—	—	Zhang *et al.*, 1996
Platelets	Yes	—	Yes	—	Hourani *et al.*, 1992; Hall and Hourani, 1993; Hechler *et al.*, 1998; Fagura *et al.*, 1998; Daniel *et al.*, 1998; Jin, *et al.*, 1998
CHO cells	Yes	Yes	—	—	Iredale and Hill, 1993
Chondrocytes	—	Yes	—	—	Kaplan *et al.*, 1996
Chromaffin cells	Yes	Yes	—	—	Reichsman *et al.*, 1995
Duct cells					
Pancreatic; cystic fibrosis	—	Yes	—	—	Chan *et al.*, 1996
Submandibular	—	Yes	—	—	Yu and Turner, 1991
Endothelium	Yes[d]	Yes	—	—	Motte *et al.*, 1993a,b; Briner and Kern, 1994; Purkiss *et al.*, 1993, 1994; Wilkinson *et al.*, 1994; Communi *et al.*, 1995; Nobles *et al.*, 1995; Miyagi *et al.*, 1996b; Ralevic and Burnstock, 1996a,b; Ralevic *et al.*, 1991b, 1997; Simonsen *et al.*, 1997
	Yes	Yes	—	Yes	Yang *et al.*, 1996
Epithelium					
Intestinal, apical; human	—	Yes	—	Yes	Inoue *et al.*, 1997
Intestinal, basolateral; human	Yes	Yes	—	—	Inoue *et al.*, 1997
Intrahepatic biliary; human	—	Yes	—	—	Wolkoff *et al.*, 1995
Mammary tumour; mouse	—	Yes	—		Enomoto *et al.*, 1994
Mammary tumour; human	Yes	Yes	—	—	Flezar and Heisler, 1993
MDCK cells; canine	Yes	Yes	—	—	Zegarra-Moran *et al.*, 1995; Firestein *et al.*, 1996; Yang *et al.*, 1997
Nasal mucosa; human	—	?[f]	—	Yes	Lazarowski *et al.*, 1997b
Ocular ciliary; human	—	Yes	—	—	Wax *et al.*, 1993
Otocyst; embryonic chick	Yes	—	—	—	Nakaoka and Yamashita, 1995
Pancreatic; human cystic fibrosis	—	Yes	—	—	Chan *et al.*, 1996; Montserrat *et al.*, 1996
Retinal pigment epithelium	—	Yes[g]	—	—	Peterson *et al.*, 1997
Tracheal; hamster	—	Yes	—	—	Abdullah *et al.*, 1996; Kim *et al.*, 1996
Tracheal; rabbit	Yes	Yes	—	—	Aksoy *et al.*, 1995
Thymic; rat	?[h]	?[h]	—	?[h]	Liu *et al.*, 1995
Submandibular salivary; mouse	Yes	Yes	—	—	Gibb *et al.*, 1994
Sweat gland; equine	Yes	Yes	—	—	Ko *et al.*, 1994
Fibroblasts	—	Yes	—	—	Fine *et al.*, 1989; Gonzalez *et al.*, 1989b,c; Marsault *et al.*, 1992; Grierson and Meldolesi, 1995a,b
Glial cells					
Enteric glia	—	Yes	—	—	Kimball and Mulholland, 1996
Bergmann glia (cerebellar)	Yes	—	—	—	Kirischuk *et al.*, 1995b
Microglia	Yes	—	—	Yes	Nörenberg *et al.*, 1997
Oligodendrocytes; cortical	Yes	—	—	—	Kirischuk *et al.*, 1995a
Oligodendrocytes; retinal	—	Yes	—	—	Kirischuk *et al.*, 1995a
Glioma					
C6/C6-2B glioma cells	Yes	Yes	—	Yes[i]	Boyer *et al.*, 1994, 1995, 1996; Munshi *et al.*, 1993; Lin and Chuang, 1994; Nicholas *et al.*, 1996; Schachter *et al.*, 1996
Neuroblastoma × glioma hybrid	Yes	Yes	—	—	Lin *et al.*, 1993; Filippov *et al.*, 1994; Reiser *et al.*, 1995
Goblet (tracheal SPOC1) cells	—	Yes	—	—	Abdullah *et al.*, 1996
Hepatocytes	Yes[d]	Yes	—	—	Charest *et al.*, 1985; Keppens and DeWulf, 1991; Keppens *et al.*, 1992; Dixon *et al.*, 1995
Keratinocytes	—	Yes	—	—	Pillai and Bikle, 1992
Kidney tubules					
Cortical thick ascending limbs	—	Yes	—	—	Paulais *et al.*, 1995
Cortical tubules	Yes	—	—	—	Cha *et al.*, 1995
Terminal inner medullary collecting duct	—	Yes	—	—	Ecelbarger *et al.*, 1994
Mesangial cells (renal)	Yes	Yes	—	—	Huwiler and Pfeilschifter, 1994; Schulze-Lohoff *et al.*, 1992, 1995; Takeda *et al.*, 1996

TABLE 13
(Continued)

	$P2Y_1$-like[a]	$P2Y_2$-like[b]	$P2Y_{ADP}$[c]	Uridine nucleotide-specific	References
Myocytes					
Cardiac	Yes	—	—	—	Qu *et al.*, 1993; Scamps and Vassort, 1994
Gastrointestinal	Yes	Yes	—	—	Blottière *et al.*, 1996; Pacaud *et al.*, 1996
Vascular	—	Yes	—	—	Erlinge *et al.*, 1995; Pacaud *et al.*, 1995; Guibert *et al.*, 1996; Malam-Souley *et al.*, 1996; Strøbæk *et al.*, 1996; Qasabian *et al.*, 1997
Osteoblasts	Yes[d]	Yes	—[j]	—	Bowler *et al.*, 1992; Sistare *et al.*, 1994, 1995; Reimer and Dixon, 1992; Gallinaro *et al.*, 1995; Dixon *et al.*, 1997b
Ovarian granulosa cells					
Human	—	Yes	—	—	Kamada *et al.*, 1994; Lee *et al.*, 1996
Porcine	Yes[d]	Yes	—[j]	—	Kamada *et al.*, 1994
Ovarian CHO cells	Yes	Yes	—	—	Iredale and Hill, 1993
Pancreatic β cells	Yes	—	—	—	Bertrand *et al.*, 1987; Hillaire-Buys *et al.*, 1994
Pheochromocytoma PC12 cells	Yes	Yes	—	—	Murrin and Boarder, 1992; Majid *et al.*, 1992, 1993; Barry and Cheek, 1994; Nikodijevic *et al.*, 1994; de Souza *et al.*, 1995; Koizumi *et al.*, 1995b
Pituitary cells					
Gonadotrophs	—	Yes	—	—	Chen *et al.*, 1994b, 1995b
Lactotrophs	—	Yes	—	—	Carew *et al.*, 1994
Salt gland cells	Yes	Yes	—	—	Martin and Shuttleworth, 1995
Schwann cells	Yes	Yes	—	—	Berti Mattera *et al.*, 1996; Ansselin *et al.*, 1997; Green *et al.*, 1997
Smooth muscle					
Gastrointestinal	—	Yes	—	—	Johnson *et al.*, 1996
Vascular	Yes	—	—	—	Kennedy and Burnstock, 1985; Mathieson and Burnstock, 1985; Burnstock and Warland, 1987; Liu *et al.*, 1989; Brizzolara and Burnstock, 1991; Keef *et al.*, 1992; Corr and Burnstock, 1994; Simonsen *et al.*, 1997
	—	Yes	—	—	Eltze and Ullrich, 1996; Miyagi *et al.*, 1996a; Malam-Souley *et al.*, 1996; Rubino and Burnstock, 1996; Qasabian *et al.*, 1997
	—	—	—	Yes	Von Kügelgen *et al.*, 1987, 1990; Saiag *et al.*, 1990, 1992; Ralevic and Burnstock, 1991b; Juul *et al.*, 1992; Lagaud *et al.*, 1996; Matsumoto *et al.*, 1997
Thyrocytes	—	Yes	—	—	Schöfl *et al.*, 1995
Trophoblastic cells (placental)	—	Yes	—	—	Petit and Belisle, 1995
Tumor cells					
Ehrlich ascites	—	Yes	—	—	Dubyak and De Young, 1985
HTC liver cell line	—	Yes	—	—	Fitz and Sostman, 1994
Osteosarcoma	Yes	—	—	—	Kumagai *et al.*, 1991

[a] $P2Y_1$-like, P2Y receptors other than $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{ADP}$, and endogenous uridine nucleotide-specific receptors; probably $P2Y_1$ receptors (based on sensitivities to 2MeSATP and/or ADP, and signalling pathways), although other P2Y subtypes cannot be excluded.
[b] $P2Y_2$-like, activated by ATP = UTP suggesting a possible identity as $P2Y_2$ receptors, although at least in rat tissues a $P2Y_4$ subtype identity cannot be excluded (as rat $P2Y_4$ receptors are activated by ATP = UTP). The possible presence of uridine nucleotide-specific receptors cannot be excluded in tissues responding to UTP.
[c] ADP-specific P2Y receptors, activated by ADP but not by ATP.
[d] Denotes ADP-specific P2Y receptors (ATP weak or inactive); note that this is also the agonist profile of $P2Y_{ADP}$ receptors.
[e] These may be the same $P2Y_1$-like receptor.
[f] The response to UTP was distinct from that to UDP, but it is not clear whether this is via actions at a $P2Y_2$- or $P2Y_4$-like receptor.
[g] UTP was five-fold more potent than ATP, thus uridine-nucleotide-specific receptors are possible.
[h] Subtype(s) not clear: stimulation of PGE_2 production by ATPγS ≥ UTP > ATP.
[i] $P2Y_6$ (Nicholas *et al.*, 1996).
[j] $P2Y_{ADP}$ receptors have been described; however, it is likely that these are ADP-specific P2Y receptors.

XVIII. Endogenous Uridine Nucleotide-Specific Receptors

The inclusion of this as a separate section is a reflection of the current lack of information about the correlation between cloned ($P2Y_4$ and $P2Y_6$) and endogenous uridine nucleotide-specific receptors. It is not intended to imply that these receptors are different, although this is a possibility. The existence of $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors identifies two receptors that can be activated by UTP ($P2Y_2$, $P2Y_4$) and one that can be activated by UDP ($P2Y_6$). Thus, it is not always clear which of these receptors mediates uridine nucleotide-mediated responses in cells and tissues. Additional complications are introduced by the coexistence of P2 receptors, the lack of selective agonists and antagonists, and the interconversion and degradation of agonists leading to contamination of solutions and to the possibility of obtaining false positive as well as negative results. With hindsight, some characterization of endogenous uridine nucleotide-specific responses in many tissues might have been achieved by more complete information on agonist activity profiles, specifically giving information about their UTP/UDP selectivity. It would be worthwhile to re-evaluate the pharmacological profile of biological tissues in light of new information on these P2Y receptors.

A. Signal Transduction Mechanisms

A uridine nucleotide-specific receptor in C6–2B rat glioma cells mediates pertussis toxin-sensitive activa-

tion of PLC and an increase in IP_3 by UTP and UDP, but is not activated by ATP and ADP (Lazarowski and Harden, 1994). The uridine nucleotide-specific receptor in RAW 264.7 macrophages is coupled to pertussis toxin-sensitive and -insensitive G proteins that mediate activation of phospholipase A_2 (PLA_2) and PLC, respectively (Lin and Lee, 1996).

B. Agonists and Antagonists

Uridine nucleotide-specific receptors are activated by UTP and/or UDP, but are not activated or only weakly activated by ATP, ADP, 2MeSATP, and α,β-meATP.

There are no selective antagonists at uridine nucleotide-specific receptors. In general, responses are insensitive to P2 receptor antagonists. However, suramin and reactive blue 2 have been reported to block the UTP-specific inositol phosphate response of RAW 264.7 macrophages (Lin and Lee, 1996).

C. Distribution and Biological Effects

Uridine nucleotide-specific receptors, suggested to be $P2Y_6$ receptors, have been described on C6–2B cells where they coexist with $P2Y_1$-like and $P2Y_2$-like receptors (Boyer *et al.*, 1993). Uridine nucleotide-specific receptors are also found on macrophages (Lin and Lee, 1996) and microglial cells (Nörenberg *et al.*, 1997a). They have been shown to mediate metabolic effects, membrane ion fluxes, and hemodynamic effects in perfused rat liver (Haussinger *et al.*, 1987). Uridine nucleotide-specific receptors mediating Cl^- secretion on human nasal mucosal (Lazarowski *et al.*, 1997b) and intestinal epithelial cells (Inoue *et al.*, 1997) are activated by UDP, perhaps indicating that these are $P2Y_6$ receptors.

Uridine nucleotide-specific receptors are found on vascular endothelium and smooth muscle. A pertussis toxin-sensitive uridine nucleotide-specific receptor coexists with $P2Y_2$-like and $P2Y_1$-like receptors on guinea-pig cardiac endothelial cells (Yang *et al.*, 1996). Uridine nucleotide-specific receptors mediating contractile responses to UTP (but not to ATP) have been described on vascular smooth muscle (Von Kügelgen *et al.*, 1987, 1990; Saiag *et al.*, 1990, 1992; Ralevic and Burnstock, 1991b; Juul *et al.*, 1992; Lagaud *et al.*, 1996). These receptors are resistant to desensitization by α,β-meATP and/or do not show cross-tachyphylaxis with responses to ATP and/or are unaffected by antagonists including PPADS and suramin. It is possible that these correspond to human $P2Y_4$ receptors. In canine epicardial coronary arteries, vasoconstriction mediated by UTP and UDP at P2Y receptors does not cross-desensitize and is distinct from vasoconstriction mediated by ATP (Matsumoto *et al.*, 1997); this suggests effects mediated at uridine nucleotide-specific receptors similar or identical with human $P2Y_4$ and $P2Y_6$ receptors, respectively.

A uridine nucleotide-specific receptor has been described in neurons of the rat superior cervical ganglion (SCG) (Boehm *et al.*, 1995; Connolly, 1995; Connolly and Harrison, 1995a, b). This receptor is activated by UTP and UDP but not by ATP, causing depolarization and transmitter release. Suramin does not block this SCG receptor (Connolly and Harrison, 1995b).

The approximately 5-fold greater potency of UTP, compared with ATP in elevating intracellular Ca^{2+} in early embryonic chick neural retina, may suggest the involvement of a uridine nucleotide-specific receptor, although the authors of this study conclude that a $P2Y_2$-like (P_{2U}) receptor is involved (Sugioka *et al.*, 1996). It is also possible that a combination of coexpressed P2Y receptors mediate this response. The biological significance of uridine nucleotide-specific receptors is unknown, but may imply differential release of purines and pyrimidines.

XVIV. $P2Y_{ADP}$ (or P_{2T}) Receptor

The $P2Y_{ADP}$ (or P_{2T}) receptor is activated by ADP, whereas ATP is a competitive antagonist. Because this receptor has not yet been cloned from the platelets or megakaryoblastic cells in which it is expressed, the recommendation of the IUPHAR committee is that the name of this receptor is written in italics. It has been suggested that the $P2Y_{ADP}$ receptor is equivalent to the $P2Y_1$ receptor based on their similar pharmacological profiles and the fact that $P2Y_1$ receptor mRNA is present in platelets and megakaryoblastic cells lines (Léon *et al.*, 1997). Although this seemed an attractive hypothesis with which to explain the enigma of the $P2Y_{ADP}$ (or P_{2T}) receptor, there is now convincing pharmacological evidence that the $P2Y_{ADP}$ (or P_{2T} receptor) is not equivalent to the $P2Y_1$ receptor; both of these receptors are expressed on platelets and cooperate to mediate platelet shape change and aggregation (Daniel *et al.*, 1998; Fagura *et al.*, 1998; Hechler *et al.*, 1998; Jin *et al.*, 1998). Notably, 2MeSATP is a full and potent agonist at the recombinant $P2Y_1$ receptor, whereas it is a noncompetitive antagonist at the $P2Y_{ADP}$ (or P_{2T}) receptor, and selective antagonists of the $P2Y_1$ receptor do not block ADP-induced inhibition of adenylate cyclase in platelets.

A. Signal Transduction Mechanisms

The $P2Y_{ADP}$ (or P_{2T}) receptor couples to a G_{i2} protein to mediate inhibition of adenylate cyclase activity (Hall and Hourani, 1993; Hourani and Hall, 1996). Conflicting reports that the $P2Y_{ADP}$ (or P_{2T}) receptor may or may not also activate PLC, generating IP_3 and elevating levels of intracellular Ca^{2+}, most likely came from observed effects of ADP at coexisting platelet $P2Y_1$ receptors. Platelet $P2Y_1$ receptors coupled to activation of PLC are now known to play a significant role in platelet shape change and cooperative aggregation with $P2Y_{ADP}$ (or P_{2T}) receptors (Daniel *et al.*, 1998; Hechler *et al.*, 1998; Jin *et al.*, 1998).

In platelets activated by ADP, rapid influx of extracellular Ca^{2+} forms a significant component of the increase in intracellular Ca^{2+}. A component of this Ca^{2+} influx seems to be caused by ADP actions on platelet $P2X_1$-like receptors (coexisting with $P2Y_{ADP}$ and $P2Y_1$ receptors) causing the opening of these nonselective cation channels (Soslau *et al.*, 1995; MacKenzie *et al.*, 1996) (also see Section IX.F.). Platelet aggregation seems to be mediated by a combination of the above pathways stimulated by $P2Y_{ADP}$ (or P_{2T} receptor), $P2Y_1$-like, and $P2X_1$-like receptor activation.

B. Desensitization

Homologous desensitization of the $P2Y_{ADP}$ (or P_{2T}) response has been observed in human erythroleukemic cells (Shi *et al.*, 1995).

C. Agonists

ADP is the archetypal agonist at $P2Y_{ADP}$ receptors. The analogs 2-chloroADP and 2-MeSADP are more potent agonists at $P2Y_{ADP}$ receptors than ADP, and ADPαS and ADPβS are partial agonists (Hall and Hourani, 1993; Hourani and Hall, 1996).

D. Antagonists

FPL 66096 (2-propylthio-d-β,γ-difluoromethylene ATP) (pA_2 8.7) (Humphries *et al.*, 1994) and ARL 67085 (formerly FPL 67085) (2-propylthio-β,γ-dichloromethylene-d-ATP) (Humphries *et al.*, 1995) are potent and selective competitive antagonists at platelet $P2Y_{ADP}$ receptors.

ATP is a competitive antagonist, with the preferred form being ATP^{4-}. The competitive effects of ATP at the $P2Y_{ADP}$ receptor may be physiologically meaningful because degradation to ADP by platelet ecto-ATPase is slow (Beukers *et al.*, 1993). 2Cl-ATP, β,γ-meATP, Ap_4A, Ap_5A, and P^1,P^6-diadenosine hexaphosphate (Ap_6A) are also competitive antagonists; 2MeSATP and adenosine are non-competitive antagonists at platelet $P2Y_{ADP}$ receptors (Harrison *et al.*, 1975; Ogilvie, 1992; Hall and Hourani, 1993). At high concentrations, Ap_3A has antithrombotic effects at the $P2Y_{ADP}$ receptor. This is in contrast with its pro-thrombotic effects at low concentrations (Ogilvie, 1992), although breakdown to ADP and adenosine may be involved. Ap_4A, Ap_5A, and Ap_6A also inhibit ADP-induced platelet aggregation, probably by competitive interaction with the $P2Y_{ADP}$ receptor (Ogilvie *et al.*, 1996). α,β-meATP and UTP are weak inhibitors of platelet aggregation (Hall and Hourani, 1993). Suramin is a non-selective antagonist at the $P2Y_{ADP}$ receptor (Hourani *et al.*, 1992; Hall and Hourani, 1993).

E. Distribution and Biological Effects

The distribution of the $P2Y_{ADP}$ receptor seems to be limited to platelets and megakaryoblastic cell lines (Vittet *et al.*, 1992; Shi *et al.*, 1995). The lack of subtype-specific agonists and antagonists apparently has led to erroneous descriptions of P_{2T} ($P2Y_{ADP}$) receptors on a number of other cell types including osteoblasts (Sistare *et al.*, 1994, 1995) and porcine ovarian granulosa cells (Kamada *et al.*, 1994); it is likely that these are in fact ADP-specific $P2Y_1$-like receptors. The P2 receptor described in porcine ovarian granulosa cells, where ATP is a competitive antagonist of ADP-induced $[Ca^{2+}]_i$ mobilization (Kamada *et al.*, 1994), may be an ADP-specific $P2Y_1$-like receptor, where ATP is a partial agonist.

A role for the platelet $P2Y_{ADP}$ receptor has been clearly defined; it mediates the aggregation of platelets to ADP during thrombosis (Born, 1962; Born and Kratzer, 1984). One source of ADP activating the $P2Y_{ADP}$ receptor may be that derived from ATP released from damaged cells in the vessel wall. The dense granules of platelets are themselves sources of high concentrations of ATP and ADP (approximately 1 M) such that platelet aggregation and degranulation leading to the release of these nucleotides is an autocatalytic process. The adenine dinucleotides Ap_3A and Ap_4A are co-stored with ADP and ATP in platelets and comprise up to 5% of the total adenine nucleotide content of the dense granules (micromolar to millimolar concentrations) (Flodgaard and Klenow, 1982; Luthje and Ogilvie, 1983; Schluter *et al.*, 1994); they are less rapidly metabolized than ATP and may have a role in the platelet aggregatory response.

Complex and cooperative signaling pathways mediated by coexisting $P2Y_{ADP}$, $P2Y_1$, and $P2X_1$ receptors seem to underlie the change in platelet shape, platelet aggregation, and secretion of dense granules to ADP. The $P2Y_1$ receptor seems to be necessary to trigger platelet shape change and aggregation (Daniel *et al.*, 1998; Hechler *et al.*, 1998; Jin *et al.*, 1998). The $P2X_1$-like receptor mediates an initial rapid influx of Ca^{2+} in platelets (MacKenzie *et al.*, 1996), which may also contribute to initiate the change in platelet shape. This Ca^{2+} influx precedes, but is independent of, the mobilization of intracellular Ca^{2+} by the $P2Y_{ADP}$ receptor (Hallam and Rink, 1985; Sage *et al.*, 1990). Mobilization of intracellular Ca^{2+} and adenylate cyclase by the $P2Y_{ADP}$ receptor seems to be linked to platelet aggregation and cooperates with effects mediated by the $P2Y_1$ receptor, such that antagonism of either receptor is sufficient to block the response. Oscillations in $[Ca^{2+}]_i$ have been described, which seem to involve the repetitive emptying and refilling of intracellular calcium stores. The mobilization of $[Ca^{2+}]_i$ seems to be required for activation of a secondary phase of Ca^{2+} influx (Sage *et al.*, 1990).

XX. Other P2Y Receptors

The following G protein-coupled receptors have been cloned and proposed as members of the P2Y receptor family. Of these, the p2y5, p2y7, p2y9, and p2y10 receptors have now been shown unequivocally not to belong to the P2Y receptor family, and the inclusion of the *Xeno-*

pus P2Y receptor ($P2Y_8$) does not seem likely as it lacks a mammalian homologue.

A. p2y5 Receptor

A receptor expressed in activated chicken T lymphocytes was proposed as a P2Y receptor based on nucleotide binding assays (Webb *et al.*, 1996b). No functional evaluation was provided. When the turkey homolog was expressed in 1321N1 human astrocytoma cells, it was shown that no signaling responses were evoked by nucleotides; this indicates that the receptor is not a member of the P2Y receptor family (Li *et al.*, 1997c). It was noted that caution should be used when interpreting the results of binding assays in the absence of robust ligands and that a prerequisite for the identification of additional P2Y receptors should be a functional demonstration of signaling responses in an appropriate cell line (Li *et al.*, 1997c).

B. p2y7/Leukotriene B_4 Receptor

It was suggested that a receptor cloned from human HEL cells was a $P2Y_7$ receptor based on binding and activation by purine nucleotides when transfected in COS-7 cells (Akbar *et al.*, 1996). However, its structure, which was noted to share 30% or less homology with other cloned P2Y receptors, has been found to be identical with that of the leukotriene B_4 receptor cloned from HL-60 cells, and sensitivity to purines can be explained by intrinsic purinoceptors ($P2Y_2$) in COS-7 cells (Yokomizo *et al.*, 1997). Expression of the putative $P2Y_7$ receptor in 1321N1 human astrocytoma cells has confirmed that this receptor is not activated by nucleotides and is not a member of the P2Y receptor family (Herold *et al.*, 1997).

C. Xenopus P2Y Receptor ($P2Y_8$)

A P2Y receptor cloned from *Xenopus* neural plate is activated equipotently by purine and pyrimidine compounds with three phosphates; ATP = UTP = ITP = CTP = GTP (Bogdanov *et al.*, 1997). The cloned receptor has a particularly long C terminal of 216 amino acids (compared with approximately 16 to 67 amino acids of other P2Y receptors) that contributes to the greater length of this protein. It has been suggested that this receptor may have a role in early development of the nervous system. The receptor was tentatively named $P2Y_8$. As a mammalian homolog of this receptor has not been identified, its inclusion as a distinct subtype of the P2Y receptor family does not seem likely.

D. $P2Y_9$ and $P2Y_{10}$ Receptors

These cloned receptors, submitted to Genbank, are not nucleotide receptors.

E. $P2Y_{Ap4A}$ (or P_{2D}) Receptor

It has been proposed that there is a distinct class of purine receptor, originally termed P_{2D} ("D" for dinucleotide), which has high affinity for the diadenosine polyphosphates (Pintor *et al.*, 1993). This receptor has not yet been cloned and thus has been given the tentative name $P2Y_{Ap4A}$. It is possible that this receptor belongs to the P2Y receptor superfamily because it seems to couple to G proteins.

In rat brain synaptosomes, [^{3}H]Ap_4A and [^{3}H]ADPβS bind to high and low affinity binding sites (Pintor *et al.*, 1993). The high affinity binding sites display an agonist potency profile that is inconsistent with that of any known subtype of P_2 receptor: Ap_4A > ADPβS > β,γmeATP > α,β-meATP ≫ 2MeSATP. In rat hippocampal slices, Ap_4A and Ap_5A activate PKC (Klishin *et al.*, 1994), which suggests the coupling of the putative $P2Y_{Ap4A}$ (or P_{2D}) receptor to G proteins. However, inhibition of synaptic transmission by diadenosine polyphosphates in hippocampal slices could be inhibited by adenosine receptor antagonists (Klishin *et al.*, 1994). So far, this receptor has been described only in the CNS (Pintor *et al.*, 1993; Klishin *et al.*, 1994).

F. P3 Receptor

A distinct P3 receptor that is activated by both nucleosides and nucleotides, and is antagonized by both xanthines and α,β-meATP, has been proposed (Shinozuka *et al.*, 1988; Forsythe *et al.*, 1991). In aiming toward a unifying system of purine and pyrimidine receptor nomenclature, this receptor may need to be renamed according to the new system of purine receptor classification when further information on its structure, signal transduction mechanisms, and pharmacological profile become available. Responses mediated by ATP at the P3 receptor are independent of its breakdown to adenosine, and stable analogs of ATP are also agonists. In some respects this receptor is similar to those P1 receptors which bind ATP and its analogs (Bailey and Hourani, 1990; Hourani *et al.*, 1991; Von Kügelgen *et al.*, 1992; King *et al.*, 1996a; Piper and Hollingsworth, 1996).

In general, the P3 receptor is prejunctional. It is activated by agonists with a potency order of 2Cl-adenosine > β,γ-meATP > ATP = adenosine, as determined for inhibition of evoked release of NA from sympathetic nerves in rat tail artery (Shinozuka *et al.*, 1988). This receptor has also been described in rat vas deferens, and UTP was additionally shown to inhibit NA overflow (Forsythe *et al.*, 1991). A receptor activated by adenosine and ATP, which is blocked by α,β-meATP, mediates outward K^+ currents, and has been identified as a novel P1 receptor, may be equivalent to the P3 receptor (King *et al.*, 1996a).

Facilitation by ATP and adenosine of evoked NA release has been shown in some vascular smooth muscle. These effects are blocked by α,β-meATP and 8-SPT, but α,β-meATP is ineffective as an agonist (Miyahara and Suzuki, 1987; Zhang *et al.*, 1989; Todorov *et al.*, 1994; Ishii *et al.*, 1995); it has been suggested that this may

represent a subtype of the P3 receptor (Dalziel and Westfall, 1994).

Another distinct P3 receptor has been proposed in smooth muscle of rabbit thoracic aorta; it is activated by both adenosine and ATP, but is xanthine- and suramin-insensitive (Chinellato *et al.*, 1994).

G. P4/Diadenosine Polyphosphate-Specific Receptor

A novel receptor for diadenosine polyphosphates, distinct from the $P2Y_{Ap4A}$ (or P_{2D}) receptor, has been proposed based on a study in rat brain synaptosomes (Pintor and Miras-Portugal, 1995a). Because this receptor is not activated by ATP, the term P4 has been suggested. Increases in synaptosomal Ca^{2+} elicited by Ap_4A and Ap_5A were not blocked with suramin and methylxanthines, in contrast with the increases in Ca^{2+} evoked by ATP, α,β-meATP, and ADPβS. Furthermore, the actions of Ap_4A and ATP did not cross-desensitize, although there was homologous desensitization to Ap_5A. It has been suggested that this receptor may be an ion channel, or is coupled to a Ca^{2+} channel (Pintor and Miras-Portugal, 1995a). This receptor has not been cloned and its existence as a distinct subtype is controversial. The synthesis of diinosine polyphosphates as antagonists with some selectivity for the effects of Ap_5A in rat brain synaptosomes *versus* the effects mediated by ATP may prove useful in the characterization of dinucleotide receptors (Pintor *et al.*, 1998).

XXI. Integrated Effects of P2 Receptors

Many cells express more than one type of P2 receptor. The biological significance of this is not entirely clear but allows potential regulation of multiple effectors, fine tuning of agonist-evoked responses, and/or synergy. The quite different specificities of many P2 receptors for endogenous agonists suggest that the source and local concentration of ADP, ATP, UDP, UTP, and adenine dinucleotides may be important; more detailed information on this might provide some insight into the biological significance of P2 receptor coexistence. A number of cells seem to express more than one type of P2Y receptor: for example, $P2Y_1$- and $P2Y_2$-like receptors are expressed on cortical astrocytes, osteoblasts, hepatocytes, endothelial, and epithelial cells; $P2Y_1$- and $P2Y_2$-like and uridine nucleotide-specific receptors are expressed on cardiac endothelial cells (Yang *et al.*, 1996) (also see table 13). These receptors typically have a common signaling pathway in PLC, and downstream divergence at subsequent steps of this pathway may be important. Synergism does not seem to occur.

Differential expression and coexpression of receptors among similar cells has been shown for $P2Y_1$-like and $P2Y_2$-like receptors on individual cultured human osteoblasts (Dixon *et al.*, 1997b) and for astrocytes from the dorsal spinal cord of the rat (Ho *et al.*, 1995). Coexpression may also differ among tissues: functional studies suggest that hamster mesenteric arteries have predominantly $P2Y_2$-like receptors and few $P2Y_1$-like receptors (Ralevic and Burnstock, 1996b), whereas the converse seems to be true for piglet aorta (Martin *et al.*, 1985) and lamb small coronary arteries (Simonsen *et al.*, 1997) where UTP is a very weak agonist. However, it is possible that these receptors are expressed but are not coupled to a vasomotor response. The physiological significance of the differential expression of P2Y receptors at the level of single cells and tissues remains to be determined.

$P2X_1$-like and $P2Y_1$-like receptors coexist on the smooth muscle in some vessels; they may reciprocally control vascular tone by acting as mediators of vasoconstriction and vasodilatation, respectively. This may occur following release of ATP from the terminals of perivascular sympathetic and sensory nerves, respectively. Cooperative effects have been shown for coexisting $P2X_1$-like, $P2Y_1$–like, and $P2Y_{ADP}$ (or P_{2T}) receptors on platelets, which mediate ionotropic Ca^{2+} influx and mobilization of intracellular Ca^{2+}, respectively, to bring about changes in platelet shape and aggregation (Hourani and Hall, 1996; MacKenzie *et al.*, 1996; Daniel *et al.*, 1998; Hechler *et al.*, 1998; Jin *et al.*, 1998). $P2Y_2$- and $P2X_7$-like receptors coexist on macrophages, although the functional significance of this, if any, remains to be determined.

Receptor expression may be regulated differently under different physiological and pathophysiological conditions, thereby altering patterns of coexpression. Expression of P2 receptors on mononuclear phagocytes is regulated differently by proinflammatory cytokines, which cause rapid down-regulation of $P2X_1$-like and $P2Y_2$-like receptors, but concomittant massive up-regulation of $P2X_7$-like receptors (Dubyak *et al.*, 1996). There also is differential functional expression during development; $P2Y_1$-like receptors are expressed only in early myeloid progenitor cells, whereas $P2Y_2$-like receptors are expressed in late stage progenitor cells, and mature monocytes and neutrophils (Dubyak *et al.*, 1996; Martin *et al.*, 1997a).

Integrated effects of P2 receptors in whole tissues are considered in the next section.

XXII. Integrated Effects of Adenosine/P1 and P2 Receptors

P1/P2 receptor coexistence has been identified for many cell types; these include $P2X_7$-, A_{2A}-, A_{2B}-, and A_3-like receptors on mast cells; $P2Y_1$, $P2Y_2$, A_{2A}, and A_{2B} receptors on endothelial cells; A_1 and $P2X_1$-like receptors on smooth muscle cells; and A_{2A}, A_{2B}, $P2Y_2$-, and $P2X_2$-like receptors on PC12 cells. The functional significance of this is not entirely clear. Among other possible interactions there may be reciprocal effects, as shown for A_1 receptor-mediated inhibition and $P2Y_1$-like receptor-mediated stimulation of insulin secretion in pancreatic β-cells (Hillaire-Buys *et al.*, 1989, 1993, 1994). Activation of A_{2A} receptors inhibits ATP-induced Ca^{2+} influx

via P2X receptors in PC12 cells (Park *et al.*, 1997), indicating antagonistic interplay between these systems. Integration of purine receptor-mediated responses at the level of whole tissues is illustrated by purinergic control of blood vessel tone, which involves vasoconstrictor $P2X_1$-like and uridine nucleotide-specific receptors on vascular smooth muscle, vasodilator $P2Y_1$-like, $P2Y_2$-like, A_{2A}, and A_{2B} receptors found on smooth muscle and endothelium, and prejunctional A_1 receptors that modulate the release of neurotransmitter from perivascular nerves (fig. 12).

Normal patterns of purinergic signaling may alter dramatically under pathophysiological conditions. The net effect of purine receptors may be vasodilatation if endothelial cells are intact, but vasoconstriction will predominate if the endothelium is damaged. When endothelial cells are damaged, collagen is exposed. Platelets adhere to the collagen and release ADP, ATP, UTP, and adenine dinucleotides, together with other substances such as 5-HT. Several substances promote further aggregation via activation of platelet $P2X_1$-like, $P2Y_1$-like, and $P2Y_{ADP}$ receptors. Purines and pyrimidines released from platelets can also act on endothelial and/or vascular smooth muscle cell P2 receptors. In an inflammatory reaction, ATP may be released from sensory nerves to have effects on mast cell $P2X_7$-like receptors, although its breakdown product adenosine may activate coexisting mast cell A_3 receptors, leading to further effects on vascular tone after release of mast cell mediators.

Understanding how responses mediated by purine receptors are integrated in biological systems depends on information on the sources of the natural agonists, as well as on the receptor signaling pathways. In addition, the metabolic relationship between purines, whereby extracellular ATP is rapidly catabolized to ADP and adenosine has important implications for colocalized adenosine/P1 and P2 receptors as there may be an interplay between these receptors. Notably, many of the above studies are concerned with short-term interactions between coexisting purine receptors, which represents only one aspect of purine and pyrimidine receptor signaling. Particularly for metabotropic G protein-coupled receptors, long-term trophic interactions are likely to be important (Cowen *et al.*, 1991; Abbracchio *et al.*, 1995b; Neary, 1996) and may lead to further insights into the significance of P1/P2 receptor coexistence and the cross-talk that may occur between these receptors. Further information awaits the development of selective agonists and antagonists and studies with genetic "knockout" animals.

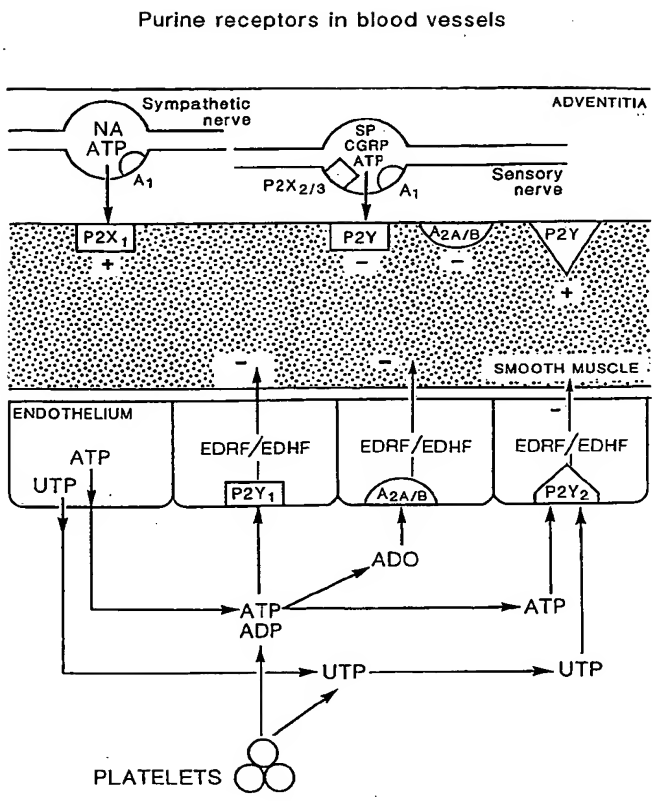

FIG. 12. Schematic of integrated effects of P1 and P2 purine receptors in the local control of vascular tone. Noradrenaline (NA), ATP, calcitonin gene-related peptide (CGRP), and substance *P* (SP) can be released from nerves in the adventitia to act on their respective receptors in the smooth muscle, causing vasoconstriction or vasodilatation. Prejunctional A_1 receptors modulate the release of neurotransmitter from sympathetic and sensory afferents. $P2X_{2/3}$ heteromers, possibly together with the corresponding homomeric P2X receptors, may be present on the peripheral terminals of sensory nerves where they may modulate sensory neurotransmission. Vasoconstriction following ATP release from perivascular nerves is mediated predominantly by $P2X_1$ receptors on the smooth muscle, while vasodilatation is mediated by smooth muscle P_{2Y} receptors ($P2Y_1$-like). P2Y receptors (possibly $P2Y_2$, $P2Y_4$, or $P2Y_6$) are also present on some vascular smooth muscle and mediate vasoconstriction to purines and pyrimidines of currently undetermined source. Vasodilatation may also be mediated by smooth muscle A_{2A} and A_{2B} adenosine receptors. ATP and its breakdown product ADP, and UTP, can be released from endothelial cells by shear stress or hypoxia, to act on endothelial $P2Y_1$ and $P2Y_2$ receptors to mediate relaxation mainly via endothelium-derived relaxing factor (EDRF, or nitric oxide) or endothelium-derived hyperpolarizing factor (EDHF). ATP can be broken down rapidly to adenosine, which may act on endothelial and smooth muscle A_{2A} and A_{2B} receptors to mediate vasodilatation. (Adapted from Burnstock, 1990).

XXIII. Conclusions

In this review we have considered in detail the pharmacological actions and interactions of purines and pyrimidines in different cells and tisssues. These are presented within a framework intended to facilitate comparison between cloned and endogenous receptors and, thereby, to promote the development of the unifying system of nomenclature based on cloned receptors. For adenosine/P1 receptors, the availability of potent and selective pharmacological ligands has been crucial in the subclassification of this family into four subtypes. For P2 receptors, responses of biological tissue have been described that do not correspond well with those of any cloned P2 receptors; there are diverse reasons, including the fact that small differences in molecular structure of a receptor are commonly found between

species and tissues and may profoundly influence its properties. Other reasons include differences in assay conditions and because coexpression of different subtypes of receptors for purines and pyrimidines is common, which leads to complex pharmacological profiles. The lack of subtype-selective agonists and antagonists with which to adequately discriminate between responses is a significant handicap. Furthermore, while we have a reasonably good idea of the properties of homomeric recombinant P2X receptors, the relative contribution of individual subunits to responses mediated by heteromeric receptors is less clear. Although G protein-coupled P2Y receptors are single membrane-spanning proteins, diversity may be introduced by alternate G protein and/or second-messenger coupling.

Major advances in adenosine/P1 receptor research in the last few years include an increased understanding of the mechanisms underlying desensitization and neuro- and cardiac-protection, therefore offering novel approaches for pharmacological manipulation of receptor activity in disease. Much still needs to be learned about the A_{2B} receptor, and the development of selective agonists and antagonists is urgently needed. As A_{2A} and A_{2B} receptors are often coexpressed by the same cell, this would promote investigations into short-term cross-talk and the long-term functional relationship between these subtypes. Newly developed ligands at the A_3 receptor will provide insights into the significance of its relatively restricted distribution and will increase our understanding of its dual protective and toxic effects. While it has long been appreciated that the different adenosine/P1 receptor subtypes have different affinities for adenosine, the fact that a single subtype can mediate opposite effects depending on its level of activation is a relatively new concept and an exciting area for further investigation. Little is known about the integrated patterns of events arising from differential activation and desensitization or up-regulation of coexisting receptors under conditions of different concentrations of adenosine, and this may be an important area for future research.

There has been a tremendous interest in the P2 receptor research in the last decade and many exciting issues have been raised. Specific questions of interest include the physiological significance of cation and pH modulation of P2X receptor activity, the true species of ATP that is the active ligand at P2 receptors, the mechanism of desensitization of P2X receptors, and the biological significance of a receptor that is activated equipotently by ATP and UTP ($P2Y_2$ and some $P2Y_4$ receptors). We expect the future will see important developments in research on receptors for pyrimidine nucleotides and investigations into the role of diadenosine polyphosphates as extracellular signaling molecules. Questions raised about the separate identity of the putative P3 receptor, and the P_{2D} and P4 receptors claimed for adenine dinucleotides, currently identified solely by their distinct pharmacology, are also likely to be resolved. Identification of novel splice variants may add significantly to the repertoire of P2 receptor-mediated responses. It is interesting that no receptors acting as ion channels, selective for extracellular pyrimidines, have been described, which is perhaps surprising given that some parallels exist for the putative extracellular roles of purines and pyrimidines. Given the widespread distribution of receptors responsive to UTP, characterization of the sources and conditions which mediate UTP release is important; there is no evidence for UTP release as a neurotransmitter to date, but it has been shown to modulate neurotransmission. The development of an assay for detection of nanomolar quantities of UTP is an exciting and important development in this field (Lazarowski *et al.*, 1997a).

Clearly, potent and selective agonists and antagonists are needed in purine and pyrimidine receptor research. Fortunately, groups in many universities and pharmaceutical industries are seeking to identify such ligands and, with the aid of high throughput screening, there is a good possibility that these and other questions will be answered in the not too distant future. The possibility of developing a transgenic animal model in which the animal P1 or P2 receptor subtype is replaced with the human homologue has been raised as a possible means of examining the function and pharmacology of the human receptor in biological tissue, with the intent of developing therapeutic strategies for human disease.

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REFERENCES

Abbracchio MP, Brambilla R, Ceruti S, Kim HO, Von Lubitz DK, Jacobson KA and Cattabeni F (1995a) G protein-dependent activation of phospholipase C by adenosine A_3 receptors in rat brain. *Mol Pharmacol* **48**:1038–1045.

Abbracchio MP and Burnstock G (1994) Purinoceptors: Are there families of P2X and P_{2Y} purinoceptors? *Pharmacol Ther* **64**:445–475.

Abbracchio MP, Ceruti S, Burnstock G and Cattabeni F (1995b) Purinoceptors on glial cells of the central nervous system: Functional and pathological implications, in *Adenosine and Adenine Nucleotides: From Molecular Biology to Integrative Physiology* (Belardinelli L and Pelleg A eds) pp 271–280, Kluwer Academic Press, Norwell.

Abbracchio MP, Fogliatto G, Paoletti AM, Rovati GE and Cattabeni F (1992) Prolonged exposure of rat brain slices to adenosine analogs: Selective desensitization of adenosine A_1 but not A_2 receptors. *Eur J Pharmacol* **227**:317–324.

Abdullah LH, Davis SW, Burch L, Yamauchi M, Randell SH, Nettesheim P and Davis CW (1996) P_{2U} purinoceptor regulation of mucin secretion in SPOC1 cells, a goblet cell line from the airways. *Biochem J* **316**:943–951.

Abe Y, Sorimachi M, Itoyama Y, Furukawa K and Akaike N (1995) ATP responses in the embryo chick ciliary ganglion cells. *Neuroscience* **64**:547–551.

Abebe W, Makujina SR and Mustafa SJ (1994) Adenosine receptor-mediated relaxation of porcine coronary artery in presence and absence of endothelium. *Am J Physiol* **266**:H2018–H2025.

Agmon Y, Dinour D and Brezis M (1993) Disparate effects of A_1- and A_2-receptor agonists on intrarenal blood flow. *Am J Physiol* **265**:F802–F806.

Ainz LF, Salgado C, Gandarias JM, Gomez R, Vallejo A and Gil-Rodrigo CE (1993) $P_1(A_2/R_a)$-purinoceptors may mediate the stimulatory effect of adenosine and adenosine analogs on acid formation in isolated rabbit parietal cells. *Pharmacol Res* **27**:319–334.

Akatsuka Y, Egashira K, Katsuda Y, Narishige T, Ueno H, Shimokawa H and Takeshita A (1994) ATP sensitive potassium channels are involved in adenosine A_2 receptor mediated coronary vasodilatation in the dog. *Cardiovasc Res* **28**:906–911.

Akbar GKM, Dasari VR, Webb TE, Ayyanathan K, Pillarisetti K, Sandhu AK, Athwal RS, Daniel JL, Ashby B, Barnard EA and Kunapuli SP (1996) Molecular cloning of a novel P_2 purinoceptor from human erythroleukaemic cells. *J Biol Chem* **271**:18363–18367.

Akhondzadeh S and Stone TW (1994) Interaction between adenosine and $GABA_A$ receptors on hippocampal neurons. *Brain Res* **665**:229–236.

Aksoy MO, Borenstein M, Li XX and Kelsen SG (1995) Eicosanoid production in rabbit tracheal epithelium by adenine nucleotides: Mediation by P2-purinoceptors. *Am J Respir Cell Mol Biol* **13**:410–417.

Alexander SPH, Cooper J, Shine J and Hill SJ (1996) Characterization of the human brain putative A_{2B} adenosine receptor expressed in Chinese hamster ovary (CHO. A_{2B4}) cells. *Br J Pharmacol* **119**:1286–1290.

Alexander SP, Losinski A, Kendall DA and Hill SJ (1994) A comparison of A_2 adenosine receptor-induced cyclic AMP generation in cerebral cortex and relaxation of pre-contracted aorta. *Br J Pharmacol* **111**:185–190.

Ali H, Cunha-Melo JR, Saul WF and Beaven MA (1990) Activation of phospholipase C via adenosine receptors provides synergistic signals for secretion in antigen-stimulated RBL-2H3 cells. *J Biol Chem* **265**:745–753.

Ali S, Mustafa SJ and Metzger WJ (1994a) Adenosine receptor-mediated bronchoconstriction and bronchial hyperresponsiveness in allergic rabbit model. *Am J Physiol* **266**:L271–L277.

Ali S, Mustafa SJ and Metzger WJ (1994b) Adenosine-induced bronchoconstriction and contraction of airway smooth muscle from allergic rabbits with late-phase airway obstruction: Evidence for an inducible adenosine A_1 receptor. *J Pharmacol Exp Ther* **268**:1328–1334.

Allgaier C, Pullmann F, Schobert A, Von Kügelgen I and Hertting G (1994a) P_2 purinoceptors modulating noradrenaline release from sympathetic neurons in culture. *Eur J Pharmacol* **252**:R7–R8.

Allgaier C, Schobert A, Belledin M, Jackish R and Hertting G (1994b) Modulation of electrically-evoked [^{3}H]-noradrenaline release from cultured chick sympathetic neurons. *Naunyn-Schmiedeberg's Arch Pharmacol* **350**:258–266.

Allgaier C, Wellmann H, Schobert A and Von Kügelgen I (1995a) Cultured chick sympathetic neurons: Modulation of electrically evoked noradrenaline release by P_2-purinoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **352**:17–24.

Allgaier C, Wellmann H, Schobert A, Kurz G and Von Kügelgen I (1995b) Cultured chick sympathetic neurons: ATP-induced noradrenaline release and its blockade by nicotinic receptor antagonists. *Naunyn-Schmiedeberg's Arch Pharmacol* **352**: 25–30.

Altiok NA, Balmforth AJ and Fredholm BB (1992) Adenosine receptor induced cAMP changes in D384 astrocytoma cells and the effect of bradykinin theron. *Acta Physiol Scand* **144**:55–63.

Anand-Srivastava MB, Cantin M, Ballak M and Picard S (1989) Desensitization of the stimulatory A_2 adenosine receptor-adenylate cyclase system in vascular smooth muscle cells from rat aorta. *Mol Cell Endocrinol* **62**:273–279.

Ansselin AD, Davey DF and Allen DG (1997) Extracellular ATP increases intracellular calcium in cultured adult Schwann cells. *Neuroscience* **76**:947–955.

Arend LJ, Handler JS, Rhim JS, Gusovsky F and Spielman WS (1989) Adenosine-sensitive phosphoinositide turnover in a newly established renal cells line. *Am J Physiol* **256**:F1067–F1074.

Arima M, Ueda S, Matsushita S, Ozawa T and Yamaguchi H (1994) Adenosine induces Cl^- efflux in endothelial cells via a pertussis toxin-sensitive G protein. *Biochem Biophys Res Commun* **204**:1143–1149.

Armstrong S and Ganote CE (1994) Adenosine receptor specificity in preconditioning of isolated rabbit cardiomyocytes: Evidence of A_3 receptor involvement. *Cardiovasc Res* **28**:1049–1056.

Armstrong S and Ganote CE (1995) In vitro ischaemic preconditioning of isolated rabbit cardiomyocytes: Effects of selective adenosine receptor blockade and calphostin C. *Cardiovasc Res* **29**:647–652.

Asimakis GK, Inners-McBride K and Conti VR (1993) Attenuation of postischaemic dysfunction by ischaemic preconditioning is not mediated by adenosine in the isolated rat heart. *Cardiovasc Res* **27**:1522–1530.

Auchampach JA, Jin X, Wan TC, Caughey GH and Linden J (1997a) Canine mast cell adenosine receptors: Cloning and expression of the A_3 receptor and evidence that degranulation is mediated by the A_{2B} receptor. *Mol Pharmacol* **52**: 846–860.

Auchampach JA, Rizvi A, Qiu Y, Tang X-L, Maldonado C, Teschner S and Boli R (1997b) Selective activation of A_3 adenosine receptors with N^6-(3-iodobenzyl)adenosine-5′-*N*-methyluronamide protects against myocardial stunning and infarction without hemodynamic changes in conscious rabbits. *Circ Res* **80**:800–809.

Ayyanathan K, Webb TE, Sandhu AK, Athwal RS, Barnard EA and Kunapuli S (1996) Cloning and chromosomal localization of the human $P2Y_1$ purinoceptor. *Biochem Biophys Res Commun* **218**:783–788.

Bailey SJ, Hickman D and Hourani SMO (1992) Characterization of the P_1-purinoceptors mediating contraction of the rat colon muscularis mucosae. *Br J Pharmacol* **105**:400–404.

Bailey SJ and Hourani SMO (1990) A study of the purinoceptors mediating contraction in the rat colon. *Br J Pharmacol* **100**:753–756.

Bailey SJ and Hourani SMO (1994) Differential effects of suramin on P_2-purinoceptors mediating contractions of the guinea-pig vas deferens and urinary bladder. *Br J Pharmacol* **112**:219–225.

Bailey SJ and Hourani SMO (1995) Effects of suramin on contractions of the guinea-pig vas deferens induced by analogs of adenosine 5′-triphosphate. *Br J Pharmacol* **114**:1125–1132.

Balachandran C and Bennett MR (1996) ATP-activated cationic and anionic conductances in cultured rat hippocampal neurons. *Neurosci Lett* **204**:73–76.

Balboa MA, Firestein BG, Godson C, Bell KS and Insel PA (1994) Protein kinase C mediates phospholipase D activation by nucleotides and phorbol esters in Madin-Darby canine kidney cells. *J Biol Chem* **269**:10511–10516.

Balcar VJ, Li Y, Killinger S and Bennett MR (1995) Autoradiography of P_{2X} ATP receptors in the rat brain. *Br J Pharmacol* **115**:302–306.

Balwierczak JL, Sharif R, Krulan CM, Field FP, Weiss GB and Miller MJS (1991) Comparative effects of a selective adenosine A_2 receptor agonist, CGS 21680, and nitroprusside in vascular smooth muscle. *Eur J Pharmacol* **196**:117–123.

Barajas-López C, Espinosa-Luna R and Gerzanich V (1994) ATP closes a potassium channel and opens a cationic conductance through different receptors in neurons of guinea pig submucous plexus. *J Pharmacol Exp Ther* **258**:1396–1402.

Barajas-López C, Muller MJ, Prieto-Gómez B and Espinosa-Luna R (1995) ATP inhibits the synaptic release of acetylcholine in submucosal neurons. *J Pharmacol Exp Ther* **274**:1238–1245.

Baraldi PG, Cacciari B, Spalluto G, Pineda de las Infantas y Villatoro MJ, Zocchi C, Dionisotti S and Ongini E (1996) Pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine derivatives: Potent and selective A_{2A} adenosine antagonists. *J Med Chem* **39**: 1164–1171.

Barbhaiya H, McClain R, Ijzerman A and Rivkees SA (1996) Site directed mutagenesis of the human A_1 adenosine receptor: Influences of acidic and hydroxy residues in the first four transmembrane domains on ligand binding. *Mol Pharmacol* **50**:1635–1642.

Bardoni R, Goldstein PA, Lee J, Gu JG and MacDermott AB (1997) ATP P2X receptors mediate fast synaptic transmission in the dorsal horn of the rat spinal cord. *J Neurosci* **17**:5297–5304.

Barnard EA, Burnstock G and Webb TE (1994) G protein-coupled receptors for ATP and other nucleotides: A new receptor family. *Trends Pharmacol Sci* **15**:67–70.

Barraco RA, Clough-Helfman C, Goodwin BP and Anderson GF (1995) Evidence for presynaptic adenosine A_{2a} receptors associated with norepinephrine release and their desensitization in the rat nucleus tractus solitarius. *J Neurochem* **65**:1604–1611.

Barraco RA, Martens KA, Parizon M and Normile HJ (1993) Adenosine A_{2a} receptors in the nucleus accumbens mediate locomotor depression. *Brain Res Bull* **31**:397–404.

Barraco RA, Martens KA, Parizon M and Normile HJ (1994) Role of adenosine A_{2a} receptors in the nucleus accumbens. *Prog Neuro-Psychopharmacol* **18**:545–553.

Barrett RJ and Droppleman DA (1993) Interactions of adenosine A_1 receptor-mediated renal vasoconstriction with endogenous nitric oxide and ANG II. *Am J Physiol* **265**:F651–F659.

Barrington WW, Jacobson KA, Hutchison AJ, Williams M and Stiles GL (1989) Identification of the A_2 adenosine receptor binding subunit by photoaffinity crosslinking. *Proc Natl Acad Sci USA* **86**:6572–6576.

Barry VA and Cheek TR (1994) Extracellular ATP triggers two functionally distinct calcium signalling pathways in PC12 cells. *J Cell Sci* **107**:451–462.

Bean BP (1992) Pharmacology and electrophysiology of ATP-activated ion channels. *Trends Pharmacol Sci* **13**:87–90.

Beindl W, Mitterauer T, Hohenegger M, Ijzerman AP, Nanoff C and Freissmuth M (1996) Inhibition of receptor/G protein coupling by suramin analogs. *Mol Pharmacol* **50**:415–423.

Belardinelli L, Shryock JC, Song Y, Wang D and Srinivas M (1995a) Ionic basis of the electrophysiological actions of adenosine on cardiomyocytes. *FASEB J* **9**:359–365.

Belardinelli L, Shryock JC, Zhang Y, Scammells PJ, Olsson R, Dennis D, Milner P, Pfister J and Baker SP (1995b) 1,3-Dipropyl-8-[2-(5,6-epoxy) norbornyl] xanthine, a potent, specific and selective A_1 adenosine receptor antagonist in the guinea pig heart and brain and in DT_1 MF-2 cells. *J Pharmacol Exp Ther* **275**:1167–1176.

Benham CD and Tsien RW (1987) A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. *Nature (Lond.)* **238**:275–278.

Berkich DA, Luthin DR, Woodward RL, Vannucci SJ, Linden J and LaNoue KF (1995) Evidence for regulated coupling of A_1 adenosine receptors by phosphorylation in Zucker rats. *Am J Physiol* **268**:E693–E704.

Berne RM (1963) Cardiac nucleotides in hypoxia: Possible role in regulation of coronary blood flow. *Am J Physiol* **204**:317–322.

Berrie CP, Hawkins PT, Stephens LR, Harden TK and Downes CP (1989) Phosphatidylinositol 4,5-bisphosphate hydrolysis in turkey erythrocytes is regulated by P_{2y} purinoceptors. *Mol Pharmacol* **35**:526–532.

Berti-Mattera LN, Wilkins PL, Madhun Z and Suchovsky D (1996) P_2-purinergic receptors regulate phospholipase C and adenylate cyclase activities in immortalized Schwann cells. *Biochem J* **314**:555–561.

Bertrand G, Chapal J, Loubatières-Mariani MM and Roye M (1987) Evidence for two different P_2-purinoceptors on beta cell and pancreatic vascular bed. *Br J Pharmacol* **91**:783–787.

Beukers MW, Kerkhof CJM, Van Rhee AM, Ardanuy U, Gurgel C, Widjaja H, Nickel P, Ijzerman AP and Soudijn W (1995) Suramin analogs, divalent cations and ATPγS as inhibitors of ecto-ATPase. *Naunyn Schmiedeberg's Arch Pharmacol* **351**:523–528.

Beukers MW, Pirovano IM, Van Weert A, Kerkhof CJ, Ijzerman AP and Soudijn W (1993) Characterization of ecto-ATPase on human blood cells: A physiological role in platelet aggregation? *Biochem Pharmacol* **46**:1959–1966.

Bhattacharya S, Dewitt DL, Burnatowska-Hledin M, Smith WL and Spielman WS (1993) Cloning of an adenosine A_1 receptor-encoding gene from rabbit. *Gene* **128**:285–288.

Biffen M and Alexander DR (1994) Mobilization of intracellular Ca^{2+} by adenine nucleotides in human T-leukaemia cells: Evidence for ADP-specific and P_{2y}-purinergic receptors. *Biochem J* **304**:769–774.

Bland-Ward PA and Humphrey PPA (1997) Acute nociception mediated by hindpaw P2X receptor activation in the rat. *Br J Pharmacol* **122**:365–371.

Blazynski C (1993) Characterization of adenosine A_2 receptors in bovine retinal pigment epithelial cells. *Exp Eye Res* **56**:595–599.

Blazynski C and McIntosh H (1993) Characterization of adenosine A_2 receptors in bovine retinal membranes. *Exp Eye Res* **56**:585–593.

Blottière HM, Loirand G and Pacaud P (1996) Rise in cytosolic Ca^{2+} concentration induced by P_2-purinoceptor activation in isolated myocytes from the rat gastrointestinal tract. *Br J Pharmacol* **117**:775–780.

Bo X and Burnstock G (1990) High- and low-affinity binding sites for [^{3}H]-α, β-methylene ATP in rat urinary bladder membranes. *Br J Pharmacol* **101**:291–296.

Bo X and Burnstock G (1993) Heterogeneous distribution of [^{3}H-α, β-MeATP binding sites in blood vessels from rat, guinea-pig, and rabbit. *J Vasc Res* **30**:87–101.

Bo X and Burnstock G (1994) Distribution of [^{3}H]α, β-methylene ATP binding sites in rat brain and spinal cord. *Neuroreport* **5**:1601–1604.

Bo X, Fischer B, Maillard M, Jacobson KA and Burnstock G (1994) Comparative studies on the affinities of ATP derivatives for P_{2X}-purinoceptors in rat urinary bladder. *Br J Pharmacol* **112**:1151–1159.

Bo X, Zhang Y, Nassar M, Burnstock G and Schöepfer R (1995) A P2X purinoceptor cDNA conferring a novel pharmacological profile. *FEBS Lett* **375**:129–133.

Bodin P, Milner P, Winter R and Burnstock G (1992) Chronic hypoxia changes the ratio of endothelin to ATP release from rat aortic endothelial cells exposed to high flow. *Proc R Soc Lond B Biol Sci* **247**:131–135.

Boehm S (1994) Noradrenaline release from rat sympathetic neurons evoked by P_2-purinoceptor activation. *Naunyn-Schmiedeberg's Arch Pharmacol* **350**:454–458.

Boehm S, Huck S and Illes P (1995) UTP- and ATP-triggered transmitter release from rat sympathetic neurons via separate receptors. *Br J Pharmacol* **116**:2341–2343.

Bogdanov Y, Dale L, King BF, Whittock N and Burnstock G (1997) Early expression of a novel nucleotide receptor in the neural plate of *Xenopus* embryos. *J Biol Chem* **272**:12583–12590.

Bogdanov YD, Wildman SS, Clements MP, King BF and Burnstock G (1998) Molecular cloning and characterisation of rat $P2Y_4$ nucleotide receptor. *Br J Pharmacol*, **124**:428–430.

Born GVR (1962) Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature (Lond.)* **194**:927–928.

Born GVR and Kratzer MAA (1984) Source and concentration of extracellular adenosine triphosphate during hemostasis in rats, rabbits and man. *J Physiol (Lond)* **354**:419–429.

Bowden A, Patel V, Brown C and Boarder MR (1995) Evidence for requirement of tyrosine phosphorylation in endothelial P_{2Y}- and P_{2U}-purinoceptor stimulation of prostacyclin release. *Br J Pharmacol* **116**:2563–2568.

Bowler WB, Birch MA, Gallagher JA and Bilbe G (1995) Identification and cloning of human P_{2U} purinoceptor present in osteoclastoma, bone, and osteoblasts. *J Bone Miner Res* **10**:1137–1145.

Boyd IA and Forrester T (1968) The release of adenosine triphosphate from frog skeletal muscle in vitro. *J Physiol (Lond)* **199**:115–135.

Boyer JL, Downes CP and Harden TK (1989) Kinetics of activation of phospholipase C by P_{2y} purinergic receptor agonists and guanine nucleotides. *J Biol Chem* **264**:884–890.

Boyer JL, Lazarowski ER, Chen X-H and Harden TK (1993) Identification of a P_{2Y}-purinergic receptor that inhibits adenylyl cyclase. *J Pharmacol Exp Ther* **267**:1140–1146.

Boyer JL, O'Tuel JW, Fischer B, Jacobson KA and Harden TK (1995) Potent agonist action of 2-thioether derivatives of adenine nucleotides at adenylyl cyclase-linked P_{2Y}-purinoceptors. *Br J Pharmacol* **116**:2611–2616.

Boyer JL, Romero-Avila T, Schachter JB and Harden TK (1996) Identification of competitive antagonists of the $P2Y_1$ receptor. *Mol Pharmacol* **50**:1323–1329.

Boyer JL, Zohn IE, Jacobson KA and Harden TK (1994) Differential effects of P_2-purinoceptor antagonists on phospholipase C- and adenylate cyclase-coupled P_{2Y}-purinoceptors. *Br J Pharmacol* **113**:614–620.

Brackett LE and Daly JW (1991) Relaxant effects of adenosine analogs on guinea pig trachea in vitro: Xanthine sensitive and xanthine-insensitive mechanisms. *J Pharmacol Exp Ther* **257**:205–213.

Brackett LE and Daly JW (1994) Functional characterization of the A_{2B} adenosine receptor in NIH 3T3 fibroblasts. *Biochem Pharmacol* **47**:801–814.

Brake AJ, Wagenbach MJ and Julius D (1994) New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature (Wash. DC)* **371**:519–523.

Brändle U, Spielmanns P, Osteroth R, Sim J, Surprenant A, Buell G, Ruppersberg JP, Plinkert PK, Zenner H-P and Glowatzki E (1997) Desensitization of the $P2X_2$ receptor controlled by alternative splicing. *FEBS Lett* **404**:294–298.

Brayden JE (1991) Hyperpolarization and relaxation of resistance arteries in response to adenosine diphosphate. *Circ Res* **69**:1415–1420.

Briejer MR, Akkermans LMA, Meulemans AL, Lefebvre RA and Schuurkes JAJ (1995) 5-HT-induced neurogenic relaxations of the guinea-pig proximal colon: Investigation into the role of ATP and VIP in addition to nitric oxide. *Naunyn-Schmiedeberg's Arch Pharmacol* **351**:126–135.

Briner VA and Kern F (1994) ATP stimulates Ca^{2+} mobilization by a nucleotide receptor in glomerular endothelial cells. *Am J Physiol* **266**:F210–F217.

Brizzolara A and Burnstock G (1991) Endothelium-dependent and endothelium-independent vasodilatation of the hepatic artery of the rabbit. *Br J Pharmacol* **103**:1206–1212.

Brizzolara AL, Crowe R and Burnstock G (1993) Evidence for the involvement of both ATP and nitric oxide in non-adrenergic non-cholinergic inhibitory neurotransmission in the rabbit portal vein. *Br J Pharmacol* **109**:606–608.

Brown C, Tanna B and Boarder MR (1995) PPADS: An antagonist at endothelial P_{2Y}-purinoceptors but not P_{2U}-purinoceptors. *Br J Pharmacol* **116**:2413–2416.

Brown SJ, James S, Reddington M and Richardson PJ (1990) Both A_1 and A_{2a} purine receptors regulate striatal acetylcholine release. *J Neurochem* **55**:31–38.

Bruns RF, Fergus JH, Badger EW, Bristol JA, Santay LA, Hartman JD, Hayes SJ and Huang CC (1987) Binding of the A_1-selective adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine to rat brain membranes. *Naunyn-Schmiedeberg's Arch Pharmacol* **335**:59–63.

Bruns RF, Lu GH and Pugsley TA (1986) Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. *Mol Pharmacol* **29**:331–346.

Buell G, Lewis C, Collo G, North RA and Surprenant A (1996a) An antagonist-insensitive P2X receptor expressed in epithelia and brain. *EMBO (Eur Mol Biol Organ) J* **15**:55–62.

Buell G, Michel AD, Lewis C, Collo G, Humphrey PP and Surprenant A (1996b) $P2X_1$ receptor activation in HL60 cells. *Blood* **87**:2659–2664.

Bullough DA, Magill MJ, Firestein GS and Mullane KM (1995) Adenosine activates A_2 receptors to inhibit neutrophil adhesion and injury to isolated cardiac myocytes. *J Immunol* **155**:2579–2586.

Bültmann R, Driessen B, Goncalves J and Starke K (1995) Functional consequences of nucleotide breakdown in rat vas deferens: A study with Evans blue. *Naunyn Schmiedeberg's Arch Pharmacol* **351**:555–560.

Bültmann R, Dudeck O and Starke K (1996) Evaluation of P_2-purinoceptor antagonists at two relaxation-mediating P_2-purinoceptors in guinea-pig taenia coli. *Naunyn-Schmiedeberg's Arch Pharmacol* **353**:445–451.

Bültmann R, Pause B, Wittenburg H, Kurz G and Starke K (1996a) P_2-purinoceptor antagonists: I. Blockade of P_2-purinoceptor subtypes and ecto-nucleotidases by small aromatic isothiocyanato-sulphonates. *Naunyn-Schmiedeberg's Arch Pharmacol* **354**:481–490.

Bültmann R and Starke K (1993) Evans blue blocks P_{2X}-purinoceptors in rat vas deferens. *Naunyn Schmiedeberg's Arch Pharmacol* **348**:684–687.

Bültmann R and Starke K (1994a) P_2-purinoceptor antagonists discriminate three contraction-mediating receptors for ATP in rat vas deferens. *Naunyn-Schmiedeberg's Arch Pharmacol* **349**:74–80.

Bültmann R and Starke K (1994b) Blockade by 4,4'-diisothiocyanatostilbene (DIDS) of P_{2X}-purinoceptors in rat vas deferens. *Br J Pharmacol* **112**:690–699.

Bültmann R and Starke K (1995) Reactive red 2: A P_{2y}-selective purinoceptor antagonist and an inhibitor of ecto-nucleotidase. *Naunyn-Schmiedeberg's Arch Pharmacol* **352**:477–482.

Bültmann R, Trendelenburg M and Starke K (1994) Blockade of P_{2X}-purinoceptors by trypan blue in rat vas deferens. *Br J Pharmacol* **113**:349–354.

Bültmann R, Wittenburg H, Pause B, Kurz G, Nickel P and Starke K (1996b) P_2-purinoceptor antagonists: III. Blockade of P_2-purinoceptor subtypes and ecto-nucleotidases by compounds related to suramin. *Naunyn-Schmiedeberg's Arch Pharmacol* **354**:498–504.

Bünemann M and Pott L (1995) Down-regulation of A_1 adenosine receptors coupled to muscarinic K^+ current in cultured guinea-pig atrial myocytes. *J Physiol (Lond)* **482**:81–95.

Burnstock G (1978) A basis for distinguishing two types of purinergic receptor, in *Cell Membrane Receptors for Drugs and Hormones* (Bolis L and Straub RW eds) pp 107–118, Raven Press, New York.

Burnstock G (1990) The fifth Heymans memorial lecture-Ghent, February 17, 1990: Co-transmission. *Arch Int Pharmacodyn Ther* **304**:7–33.

Burnstock G, Fischer B, Hoyle CHV, Maillard M, Ziganshin AU, Brizzolara AL, Von Isakovics A, Boyer JL, Harden TK and Jacobson KA (1994) Structure activity relationships for derivatives of adenosine 5'-triphosphate as agonists at P_2 purinoceptors: Heterogeneity within P_{2X} and P_{2Y} subtypes. *Drug Dev Res* **31**:206–219.

Burnstock G, Hills JM and Hoyle CHV (1984) Evidence that the P_1-purinoceptor in the guinea-pig taenia coli is an A_2-subtype. *Br J Pharmacol* **81**:533–541.

Burnstock G and Kennedy C (1985) Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen Pharmacol* **16**:433–440.

Burnstock G and Kennedy C (1986) Purinergic receptors in the cardiovascular system. *Prog Pharmacol* **6**:111–132.

Burnstock G and King BF (1996) Numbering of cloned P2 purinoceptors. *Drug Dev Res* **38**:67–71.

Burnstock G and Ralevic V (1996) Cotransmission, in *The Pharmacology of Vascular Smooth Muscle* (Garland CJ and Angus J eds) pp 210–232, Oxford University Press, Oxford.

Burnstock G and Warland JJ (1987a) P_2-Purinoceptors of two subtypes in the rabbit mesenteric artery: Reactive blue 2 selectively inhibits responses mediated via the P_{2Y}- but not the P_{2X}-purinoceptor. *Br J Pharmacol* **90**:383–391.

Burnstock G and Warland JJ (1987b) A pharmacological study of the rabbit saphenous artery in vitro: A vessel with a large purinergic contractile response to sympathetic nerve stimulation. *Br J Pharmacol* **90**:111–120.

Burnstock G and Wood JN (1996) Purinergic receptors: Their role in nociception and primary afferent neurotransmission. *Curr Opin Neurobiol* **6**:526–532.

Buxton DB, Fisher RA, Robertson SM and Olson MS (1987) Stimulation of glycogenolysis and vasoconstriction by adenosine and adenosine analogs in the perfused rat liver. *Biochem J* **248**:35–41.

Camaioni E, Boyer JL, Mohanram A, Harden TK and Jacobson KA (1998) Deoxyadenosine bisphosphate derivatives as potent antagonists at $P2Y_1$ receptors. *J Med Chem* **41**:183–190.

Carew MA, Wu ML, Law GL, Tseng YZ and Mason WT (1994) Extracellular ATP activates calcium entry and mobilization via P_{2U}-purinoceptors in rat lactotrophs. *Cell Calcium* **16**:227–235.

Carruthers AM and Fozard JR (1993) Adenosine A_3 receptors: Two into one won't go. *Trends Pharmacol Sci* **14**:290–291.

Casado V, Casillas T, Mallol J, Canela EI, Lluis C and Franco R (1992) The adenosine receptors present on the plasma membrane of chromaffin cells are of the A_{2b} subtype. *J Neurochem* **59**:425–431.

Casati C, Monopoli A, Dionisotti S, Zocchi C, Bonizzoni E and Ongini E (1994) Repeated administration of selective adenosine A_1 and A_2 receptor agonists in the spontaneously hypertensive rat: Tolerance develops to A_1-mediated hemodynamic effects. *J Pharmacol Exp Ther* **268**:1506–1511.

Casavola V, Guerra L, Reshkin SJ, Jacobson KA and Murer H (1997) Polarization of adenosine effects on intracellular pH in A_6 renal epithelial cells. *Mol Pharmacol* **51**:516–523.

Castillo-Melendez M, Krstew E, Lawrence AJ and Jarrott B (1994) Presynaptic adenosine A_{2a} receptors on soma and central terminals of rat vagal afferent neurons. *Brain Res* **652**:137–144.

Castro E, Mateo J, Tome AR, Barbosa RM, Miras-Portugal MT and Rosario LM (1995) Cell-specific purinergic receptors coupled to Ca^{2+} entry and Ca^{2+} release from internal stores in adrenal chromaffin cells: Differential sensitivity to UTP and suramin. *J Biol Chem* **270**:5098–5106.

Cena V and Rojas E (1990) Kinetic characterization of calcium-dependent, cholinergic receptor-controlled ATP secretion from adrenal medullary chromaffin cells. *Biochim Biophys Acta* **1023**:213–222.

Cha SH, Jung KY and Endou H (1995) Effect of P_{2Y}-purinoceptor stimulation on renal gluconeogenesis in rats. *Biochem Biophys Res Commun* **211**:454–461.

Chahwala SB and Cantley LC (1984) Extracellular ATP induces ion fluxes and inhibits growth of friend erythroleukemia cells. *J Biol Chem* **259**:13717–13722.

Challis RAJ, Richards SJ and Budohoski L (1992) Characterization of the adenosine

receptor modulating insulin action in skeletal muscle. *Eur J Pharmacol* **226**:121–128.

Chan HC, Cheung WT, Leung PY, Wu LJ, Chew SB, Ko WH and Wong PY (1996) Purinergic regulation of anion secretion by cystic fibrosis pancreatic duct cells. *Am J Physiol* **271**:C469–C477.

Chang K, Hanaoka K, Kumada M and Takuwa Y (1995) Molecular cloning and functional analysis of a novel P_2 nucleotide receptor. *J Biol Chem (Tokyo)* **270**: 26152–26158.

Chapal J, Loubatières-Mariani MM, Petit P and Royal M (1985) Evidence for an A_2-subtype adenosine receptor on pancreatic glucagon secreting cells. *Br J Pharmacol* **86**:565–569.

Charest R, Blackmore PF and Exton JH (1985) Characterization of responses of isolated rat hepatocytes to ATP and ADP. *J Biol Chem* **260**:15789–15794.

Charlton SJ, Brown CA, Weisman GA, Turner JT, Erb L and Boarder MR (1996a) PPADS and suramin as antagonists at cloned P_{2Y}- and P_{2U}-purinoceptors. *Br J Pharmacol* **118**:704–710.

Charlton SJ, Brown CA, Weisman GA, Turner JT, Erb L and Boarder MR (1996b) Cloned and transfected $P2Y_4$ receptors: Characterization of a suramin and PPADS-insensitive response to UTP. *Br J Pharmacol* **119**:1301–1303.

Chen C-C, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G and Wood JN (1995a) A P_{2X} purinoceptor expressed by a subset of sensory neurons. *Nature (Lond.)* **377**:428–431.

Chen CC and Chen WC (1996) ATP-evoked inositol phosphates formation through activation of P_{2U} purinergic receptors in cultured astrocytes: Regulation by PKC subtypes α, δ, and γ. *Glia* **17**:63–71.

Chen BC, Lee C-M, Lee YT and Lin W-W (1996a) Characterization of signaling pathways of P_{2Y} and P_{2U} purinoceptors in bovine pulmonary artery endothelial cells. *J Cardiovasc Pharmacol* **28**:192–199.

Chen BC, Lee C-M and Lin WW (1996c) Inhibition of ecto-ATPase by PPADS, suramin and reactive blue 2 in endothelial cells, C_6 glioma cells and RAW 264.7 macrophages. *Br J Pharmacol* **119**:1628–1634.

Chen ZP, Kratzmeier M, Levy A, McArdle CA, Poch A, Day A, Mukhopadhyay AK and Lightman SL (1995b) Evidence for a role of pituitary ATP receptors in the regulation of pituitary function. *Proc Natl Acad Sci USA* **92**:5219–5223.

Chen ZP, Krull N, Xu S, Levy A and Lightman SL (1996b) Molecular cloning and functional characterization of a rat pituitary G protein-coupled ATP receptor. *Endocrinology* **137**:1833–1840.

Chen ZP, Levy A and Lightman SL (1994a) Activation of specific ATP receptors induces a rapid increase in intracellular calcium ions in rat hypothalamic neurons. *Brain Res* **641**:249–256.

Chen ZP, Levy A, McArdle CA and Lightman SL (1994b) Pituitary ATP receptors: Characterization and functional localization to gonadotropes. *Endocrinology* **135**: 1280–1283.

Chern Y, King K, Lai H-L and Lai HT (1992) Molecular cloning of a novel adenosine receptor gene from rat brain. *Biochem Biophys Res Commun* **185**:304–309.

Chern Y, Lai HL, Fong JC and Liang Y (1993) Multiple mechanisms for desensitization of A_{2A} adenosine receptor-mediated cAMP elevation in rat pheochromocytoma PC12 cells. *Mol Pharmacol* **44**:950–958.

Chessell LP, Michel AD and Humphrey PPA (1997) Functional evidence for multiple purinoceptor subtypes in the rat medial vestibular nucleus. *Neuroscience* **77**:783–791.

Chiang PH, Wu SN, Tsai EM, Wu CC, Shen MR, Huang CH and Chiang CP (1994) Adenosine modulation of neurotransmission in penile erection. *Br J Clin Pharmacol* **38**:357–362.

Chinellato A, Ragazzi E, Pandolfo L, Froldi G, Caparrotta L and Fassina G (1994) Purine- and nucleotide-mediated relaxation of rabbit thoracic aorta: Common and different sites of action. *J Pharm Pharmacol* **46**:337–341.

Chiozzi P, Sanz JM, Ferrari D, Falzoni S, Aleotti A, Buell GN, Collo G and Di Virgilio F (1997) Spontaneous cell fusion in macrophage cultures expressing high levels of the P2Z/$P2X_7$ receptor. *J Cell Biol* **138**:697–706.

Choi S-Y and Kim K-T (1996) Characterization of Na^+ influx mediated by ATP^{4-}-activated P_2 purinoceptors in PC12 cells. *Br J Pharmacol* **118**:935–940.

Choo LK (1981) The effect of reactive blue, an antagonist of ATP, on the isolated urinary bladders of guinea-pig and rat. *J Pharm Pharmacol* **33**:248–250.

Christofi FL, Baidan LV, Fertel RH and Wood JD (1994) Adenosine A_2 receptor-mediated excitation of a subset of AH/type 2 neurons and elevation of cAMP levels in myenteric ganglia of guinea-pig ileum. *Neurogastroenterol Motility* **6**:67–78.

Churchill PC and Bidani A (1987) Renal effects of selective adenosine receptor agonists in anaesthetized rats. *Am J Physiol* **252**:F299–F303.

Churchill PC and Churchill MC (1985) A_1 and A_2 adenosine receptor activation inhibits and stimulates renin secretion of rat renal cortical slices. *J Pharmacol Exp Ther* **232**:589–594.

Churchill PC and Ellis VR (1993a) Pharmacological characterization of the renovascular P_2 purinergic receptors. *J Pharmacol Exp Ther* **265**:334–338.

Churchill PC and Ellis VR (1993b) Purinergic P_{2y} receptors stimulate renin secretion by rat renal cortical slices. *J Pharmacol Exp Ther* **266**:160–163.

Clarke LL and Boucher RC (1992) Chloride secretory response to extracellular ATP in human normal and cystic fibrosis nasal epithelia. *Am J Physiol* **263**:C348–C356.

Cloues R (1995) Properties of ATP-gated channels recorded from rat sympathetic neurons: Voltage dependence and regulation by Zn^{2+} ions. *J Neurophysiol* **73**:312–319.

Cloues R, Jones S and Brown DA (1993) Zn^{2+} potentiates ATP-activated currents in rat sympathetic neurons. *Pflüeg Arch Eur J Physiol* **424**:152–158.

Cockcroft S and Gomperts BD (1980) The ATP^{4-} receptor of rat mast cells. *Biochem J* **188**:789–798.

Collis MG and Brown CM (1983) Adenosine relaxes the aorta by interacting with an A_2 receptor and an intracellular site. *Eur J Pharmacol* **96**:61–69.

Collis MG and Pettinger SJ (1982) Can ATP stimulate P_1-receptors in guinea-pig atrium without conversion to adenosine? *Eur J Pharmacol* **81**:521–529.

Collo G, Neidhart S, Kawashima E, Kosco-Vilbois M, North RA and Buell G (1997) Tissue distribution of the $P2X_7$ receptor. *Neuropharmacology* **36**:1277–1283.

Collo G, North RA, Kawashima E, Merlo-Pich E, Neidhart S, Surprenant A and Buell G (1996) Cloning of $P2X_5$ and $P2X_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J Neurosci* **16**:2495–2507.

Communi D, Govaerts C, Parmentier M and Boeynaems JM (1997) Cloning of human purinergic P2Y receptor coupled to phospholipase C and adenylyl cyclase. *J Biol Chem* **272**:31969–31973.

Communi D, Motte S, Boeynaems J-M and Pirotton S (1996a) Pharmacological characterization of the human $P2Y_4$ receptor. *Eur J Pharmacol* **317**:383–389.

Communi D, Parmentier M and Boeynaems J-M (1996b) Cloning, functional expression and tissue distribution of the human $P2Y_6$ receptor. *Biochem Biophys Res Commun* **222**:303–308.

Communi D, Pirotton S, Parmentier M and Boeynaems J-M (1996c) Cloning and functional expression of a human uridine nucleotide receptor. *J Biol Chem* **270**: 30849–30852.

Communi D, Raspe E, Pirotton S and Boeynaems JM (1995) Coexpression of P_{2Y} and P_{2U} receptors on aortic endothelial cells: Comparison of cell localization and signaling pathways. *Circ Res* **76**:191–198.

Connolly GP (1995) Differentiation by pyridoxal 5-phosphate, PPADS and isoPPADS between responses mediated by UTP and those evoked by α, β-methylene-ATP on rat sympathetic ganglia. *Br J Pharmacol* **114**:727–731.

Connolly GP and Harrison PJ (1994) Reactive blue 2 discriminates between responses mediated by UTP and those evoked by ATP or α, β-methylene-ATP on rat sympathetic ganglia. *Eur J Pharmacol* **259**:95–99.

Connolly GP and Harrison PJ (1995a) Discrimination between UTP- and P_2-purinoceptor-mediated depolarization of rat superior cervical ganglia by 4,4'-diisothiocyanatostilbene-2,2'-disulphonate (DIDS) and uniblue A. *Br J Pharmacol* **115**:427–432.

Connolly GP and Harrison PJ (1995b) Structure-activity relationship of a pyrimidine receptor in the rat isolated superior cervical ganglion. *Br J Pharmacol* **115**:2764–2770.

Conti A, Monopoli A, Gamba M, Borea PA and Ongini E (1993) Effects of selective A_1 and A_2 adenosine receptor agonists on cardiovascular tissues. *Naunyn-Schmiedeberg's Arch Pharmacol* **348**:108–112.

Cook SP and McCleskey EW (1997) Desensitization, recovery and Ca^{2+}-dependent modulation of ATP-gated P2X receptors in nociceptors. *Neuropharmacology* **36**: 1303–1308.

Cook SP, Vulchanova L, Hargreaves KM, Elde R and McCleskey EW (1997) Distinct ATP receptors on pain-sensing and stretch-sensing neurons. *Nature (Lond.)* **387**: 505–508.

Cornfield LJ, Hu S, Hurt SD and Sills MA (1992) [^{3}H]2-phenylaminoadenosine ([^{3}H]CV 1808) labels a novel adenosine receptor in rat brain. *J Pharmacol Exp Ther* **263**:552–561.

Corr L and Burnstock G (1994) Analysis of P_2-purinoceptor subtypes on the smooth muscle and endothelium of rabbit coronary artery. *J Cardiovasc Pharmacol* **23**: 709–715.

Correia-de-Sá P and Ribeiro JA (1994a) Evidence that the presynaptic A_{2a}-adenosine receptor of the rat motor nerve endings is positively coupled to adenylate cyclase. *Naunyn-Schmiedeberg's Arch Pharmacol* **350**:514–522.

Correia-de-Sá P and Ribeiro JA (1994b) Potentiation by tonic A_{2a}-adenosine receptor activation of CGRP-facilitated [^{3}H]-ACh release from rat motor nerve endings. *Br J Pharmacol* **111**:582–588.

Correia-de-Sá P, Timóteo MA and Ribeiro JA (1996) Presynaptic A_1 inhibitory/A_{2A} facilitatory adenosine receptor activation balance depend on motor nerve stimulation paradigm at the rat hemidiaphragm. *J Neurophysiol* **76**:3910–3919.

Cowen DS, Berger M, Nuttle L and Dubyak GR (1991) Chronic treatment with P_2-purinergic receptor agonists induces phenotypic modulation of the HL-60 and U937 human myelogenous leukemia cell lines. *J Leukocyte Biol* **50**:109–122.

Crack BE, Beukers MW, McKechnie KCW, Ijzerman AP and Leff P (1994) Pharmacological analysis of ecto-ATPase inhibition: Evidence for combined enzyme inhibition and receptor antagonism in P_{2X}-purinoceptor ligands. *Br J Pharmacol* **113**:1432–1438.

Cristalli G, Camaioni E, Vittori S, Volpini R, Borea PA, Conti A, Dionisotti S, Ongini E and Monopoli A (1995) 2-Aralkynyl and 2-heteroalkynyl derivatives of adenosine-5'-N-ethyluronamide as selective A_{2A} adenosine receptor agonists. *J Med Chem* **38**:1462–1472.

Cristalli G, Volpini R, Vittori S, Camaioni E, Monopoli A, Conti A, Dionisotti S, Zocchi C and Ongini E (1994) 2-Alkyl derivatives of adenosine-5'-N-ethyluronamide: Selective A_2 adenosine receptor agonists with potent inhibitory activity on platelet aggregation. *J Med Chem* **37**:1720–1726.

Cronstein BN (1994) Adenosine, an endogenous anti-inflammatory agent. *J Appl Physiol* **76**:5–13.

Cronstein BN, Daguma L, Nichols D, Hutchison AJ and Williams M (1990) The adenosine/neutrophil paradox resolved: Human neutrophils possess both A_1 and A_2 receptors that promote chemotaxis and inhibit O_2 generation, respectively. *J Clin Invest* **85**:1150–1157.

Cronstein BN, Levin RI, Philips M, Hirschhorn R, Abramson SB and Weissman G (1992) Neutrophil adherence to endothelium is enhanced via adenosine A_1 receptors and inhibited via adenosine A_2 receptors. *J Immunol* **148**:2201–2206.

Cunha RA, Johansson B, Constantino MD, Sebastião AM and Fredholm BB (1996) Evidence for high-affinity binding sites for the adenosine A_{2A} receptor agonist [^{3}H] CGS 21680 in the rat hippocampus and cerebral cortex that are different from striatal receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **353**:261–271.

Cunha RA, Johansson B, Fredholm BB, Ribeiro JA and Sebastião AM (1995) Adenosine A_{2A} receptors stimulate acetylcholine release from nerve terminals of the rat hippocampus. *Neurosci Lett* **196**:41–44.

Cunha RA, Milusheva E, Vizi ES, Ribeiro JA and Sebastião AM (1994) Excitatory and inhibitory effects of A_1 and A_{2A} adenosine receptor activation on the electrically evoked [^{3}H] acetylcholine release from different areas of the rat hippocampus. *J Neurochem* **63**:207–214.

Cusack NJ, Hourani SMO, Loizou GD and Welford LA (1987) Pharmacological effects of isopolar phosphonate analogs of ATP on P_2-purinoceptors in guinea-pig taenia coli and urinary bladder. *Br J Pharmacol* **90**:791–795.

Daly JW, Butts-Lamb P and Padgett W (1983) Subclasses of adenosine receptors in the central nervous system: Interactions with caffeine and related methylxanthines. *Cell Mol Neurobiol* **3**:69–80.

Daly JW and Padgett WL (1992) Agonist activity of 2- and 5'-substituted adenosine analogs and their N^6-cycloalkyl derivatives at A_1- and A_2-adenosine receptors coupled to adenylate cyclase. *Biochem Pharmacol* **43**:1089–1093.

Daly JW, Padgett WL, Secunda SI, Thompson RD and Olsson RA (1993) Structure-activity relationships for 2-substituted adenosines at A_1 and A_2 adenosine receptors. *Pharmacology* **46**:91–100.

Daly JW, Padgett W, Shamim MT, Butts-Lamb P and Waters J (1985) 1,3-Dialkyl-8-(*p*-sulfophenyl)-xanthines: Potent water-soluble antagonists for A_1- and A_2-adenosine receptors. *J Med Chem* **28**:487–492.

Dalziel HH and Westfall DP (1994) Receptors for adenine nucleotides and nucleosides: Subclassification, distribution, and molecular characterization. *Pharmacol Rev* **46**:449–466.

Damer S, Niebel B, Czeche S, Nickel P, Ardanuy U, Mutschler E and Lambrecht G (1998) A novel suramin analog displaying high selectivity for P2X-receptors. *Drug Dev Res*, **43**:28.

Daniel JL, Dangelmaier C, Jin J, Ashby B, Smith JB and Kunapuli SP (1998) Molecular basis for ADP-induced platelet activation: I. Evidence for three distinct ADP receptors on human platelets. *J Biol Chem* **273**:2024–2029.

Dart C and Standen NB (1993) Adenosine-activated potassium current in smooth muscle cells isolated from the pig coronary artery. *J Physiol (Lond)* **471**:767–786.

Dawicki DD, McGowan-Jordan J, Bullard S, Pond S and Rounds S (1995) Extracellular nucleotides stimulate leukocyte adherence to cultured pulmonary artery endothelial cells. *Am J Physiol* **268**:L666–L673.

Day TA, Sibbald JR and Khanna S (1993) ATP mediates an excitatory noradrenergic neuron input to supraoptic vasopressin cells. *Brain Res* **607**:341–344.

DeLander GE and Hopkins CJ (1987) Involvement of A_2 adenosine receptors in spinal mechanisms of antinociception. *Eur J Pharmacol* **139**:215–223.

de Mendonça A and Ribeiro JA (1997) Influence of metabotropic glutamate receptor agonists on the inhibitory effects of adenosine A_1 receptor activation in the rat hippocampus. *Br J Pharmacol* **121**:1541–1548.

Dennis DM, Shyrock JC and Belardinelli L (1995) Homologous desensitization of the A_1-adenosine receptor system in the guinea pig atrioventricular node. *J Pharmacol Exp Ther* **272**:1024–1035.

de Souza LR, Moore H, Raha S and Reed JK (1995) Purine and pyrimidine nucleotides activate distinct signalling pathways in PC12 cells. *J Neurosci Res* **41**:753–763.

Dickenson JM and Hill SJ (1994) Interactions between adenosine A_1- and histamine H_1-receptors. *Int J Biochem* **26**:959–969.

Dickenson JM and Hill SJ (1996) Synergistic interactions between human transfected adenosine A_1 receptors and endogenous cholecystokinin receptors in CHO cells. *Eur J Pharmacol* **302**:141–151.

Dickenson JM and Hill SJ (1997) Transfected adenosine A_1 receptor mediated modulation of thrombin-stimulated phospholipase C and phospholipase A_2 activity in CHO cells. *Eur J Pharmacol* **321**:77–86.

Dionisotti S, Ongini E, Zocchi C, Kull B, Arslan G and Fredholm BB (1997) Characterization of human A_{2A} adenosine receptors with the antagonist radioligand [^{3}H]SCH 58261. *Br J Pharmacol* **121**:353–360.

Dixon CJ, Bowler WB, Walsh CA and Gallagher JA (1997b) Effects of extracellular nucleotides on single cells and populations of human osteoblasts: Contribution of cell heterogeneity to relative potencies. *Br J Pharmacol* **120**:777–780.

Dixon CJ, Cobbold PH and Green AK (1995) Actions of ADP, but not ATP, on cytosolic free Ca^{2+} in single rat hepatocytes mimicked by 2-methylthioATP. *Br J Pharmacol* **116**:1979–1984.

Dixon AK, Gubitz AK, Sirinathsinghji DJS, Richardson PJ and Freeman TC (1996) Tissue distribution of adenosine receptor mRNAs in the rat. *Br J Pharmacol* **118**:1461–1468.

Dixon AK, Widdowson L and Richardson PJ (1997a) Desensitization of the adenosine A_1 receptor by the A_{2A} receptor in the rat striatum. *J Neurochem* **69**:315–321.

Dobronyi I, Hung KS, Satchell DG and Maguire MH (1997) Evidence for a novel P_{2X} purinoceptor in human placental chorionic surface arteries. *Eur J Pharmacol* **320**:61–64.

Dolphin AC, Forda SR and Scott RH (1986) Calcium-dependent currents in cultured rat dorsal root ganglion cells are inhibited by an adenosine analog. *J Physiol (Lond)* **373**:47–61.

Doyle MP, Linden J and Duling BR (1994) Nucleoside-induced arteriolar constriction: A mast cell-dependent response. *Am J Physiol* **266**:H2042–H2050.

Drury AN and Szent-Györgyi A (1929) The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J Physiol (Lond)* **68**:213–237.

Dubey RK, Gillespie DG, Osaka K, Suzuki F and Jackson EK (1996) Adenosine inhibits growth of rat aortic smooth muscle cells: Possible role of A_{2b} receptor. *Hypertension* **27**:786–793.

Dubyak GR (1991) Signal transduction by P_2-purinergic receptors for extracellular ATP. *Am J Respir Cell Mol Biol* **4**:295–300.

Dubyak GR and De Young MB (1985) Intracellular Ca^{2+} mobilization by extracellular ATP in Ehrlich ascites tumour cells. *J Biol Chem* **260**:10653–10661.

Dubyak GR and el-Moatassim C (1993) Signal transduction via P_2-purinergic receptors for extracellular ATP and other nucleotides. *Am J Physiol* **265**:C577–C606.

Dubyak AR, Clifford EE, Humphreys BD, Kertesy SB and Martin KA (1996) Expression of multiple ATP receptor subtypes during the differentiation and inflammatory activation of myeloid leukocytes. *Drug Dev Res* **39**:269–278.

Dunn PM and Blakeley AGH (1988) Suramin: A reversible P_2-purinoceptor antagonist in the mouse vas deferens. *Br J Pharmacol* **93**:243–245.

Dunwiddie TV, Diao L, Kim H, Jiang J-L and Jacobson KA (1997) Activation of hippocampal adenosine A_3 receptors produces a desensitization of A_1 receptor-mediated responses in rat hippocampus. *J Neurosci* **17**:607–614.

Ecelbarger CA, Maeda Y, Gibson CC and Knepper MA (1994) Extracellular ATP increases intracellular calcium in rat terminal collecting duct via a nucleotide receptor. *Am J Physiol* **267**:F998–F1006.

Edwards FA and Gibb AJ (1993) ATP: A fast transmitter. *FEBS Lett* **325**:86–89.

Edwards FA, Gibb AJ and Colquhoun D (1992) ATP receptor mediated synaptic currents in the central nervous system. *Nature (Lond.)* **359**:144–146.

el-Etr M, Lombes M, Baulieu EE and Erlanger BF (1992a) A monoclonal anti-idiotypic 'internal image' antibody that recognizes the A_1 adenosine receptor potentiates the α_1-adrenergic activation of phospholipase C in primary cultures of mouse striatal astrocytes. *Neurosci Lett* **145**:15–18.

el-Etr M, Marin P, Tence M, Delumeau JC, Cordier J, Glowinski J and Premont J (1992b) 2-Chloroadenosine potentiates the α_1-adrenergic activation of phospholipase C through a mechanism involving arachidonic acid and glutamate in striatal astrocytes. *J Neurosci* **12**:1363–1369.

el-Hashim A, D'Agostino B, Matera MG and Page C (1996) Characterization of adenosine receptors involved in adenosine-induced bronchoconstriction in allergic rabbits. *Br J Pharmacol* **119**:1262–1268.

Ellsworth ML, Forrester T, Ellis CG and Dietrich HH (1995) The erythrocyte as a regulator of vascular tone. *Am J Physiol* **269**:H2155–H2161.

el-Moatassim C and Dubyak GR (1992) A novel pathway for the activation of phospholipase D by P_{2z} purinergic receptors in BAC1.2F5 macrophages. *J Biol Chem* **267**:23664–23673.

el-Moatassim C and Dubyak GR (1993) Dissociation of the pore-forming and phospholipase D activities stimulated via P_{2z} purinergic receptors in BAC1.2F5 macrophages. *J Biol Chem* **268**:15571–15578.

Eltze M and Ullrich B (1996) Characterization of vascular P_2 purinoceptors in the rat isolated perfused kidney. *Eur J Pharmacol* **306**:139–152.

Emmelin N and Feldberg W (1948) Systemic effects of adenosine triphosphate. *Br J Pharmacol* **3**:273–284.

Enomoto K, Furya K, Yamagishi S, Oka T and Maeno T (1994) The increase in the intracellular Ca^{2+} concentration induced by mechanical stimulation is propagated via release of pyrophosphorylated nucleotides in mammary epithelial cells. *Pflüeg Arch Eur J Physiol* **427**:533–542.

Erb L, Garrad R, Wang Y, Quinn T, Turner JT and Weisman GA (1995) Site-directed mutagenesis of P_{2U} purinoceptors: Positively charged amino acids in transmembrane helices 6 and 7 affect agonist potency and specificity. *J Biol Chem* **270**:4185–4188.

Erb L, Lustig KD, Ahmed AH, Gonzalez FA and Weisman GA (1990) Covalent incorporation of 3'-O-(4-benzoyl)benzoyl-ATP into a P_2 purinoceptor in transformed mouse fibroblasts. *J Biol Chem* **265**:7424–7431.

Ergene E, Dunbar JC, O'Leary DS and Barraco RA (1994) Activation of P_2-purinoceptors in the nucleus tractus solitarius mediate depressor responses. *Neurosci Lett* **174**:188–192.

Erlinge D, Hou M, Webb T, Barnard E and Möller S (1998) Upregulation of mitogenic P2-receptor mRNA in the synthetic phenotype of the vascular smooth muscle cell measured by a new quantitative competitive RT-PCR. *Drug Dev Res*, **43**:5.

Erlinge D, You J, Wahlestedt C and Edvinsson L (1995) Characterisation of an ATP receptor mediating mitogenesis in vascular smooth muscle cells. *Eur J Pharmacol* **289**:135–149.

Evans RJ, Derkarch V and Surprenant A (1992) ATP mediates fast synaptic transmission in mammalian neurons. *Nature (Lond.)* **357**:503–505.

Evans RJ and Kennedy C (1994) Characterization of P_2-purinoceptors in the smooth muscle of the rat tail artery: A comparison between contractile and electrophysiological responses. *Br J Pharmacol* **113**:853–860.

Evans RJ, Lewis C, Buell G, Valera S, North RA and Surprenant A (1995) Pharmacological characterization of heterologously expressed ATP-gated cation channels (P_{2X} purinoceptors). *Mol Pharmacol* **48**:178–183.

Evans RJ, Lewis C, Virginio C, Lundstrom K, Buell G, Surprenant A and North RA (1996) Ionic permeability of, and divalent cation effects on, two ATP-gated cation channels (P2X receptors) expressed in mammalian cells. *J Physiol (Lond)* **497**: 413–422.

Evans RJ and Surprenant A (1996) P2X receptors in autonomic and sensory neurons. *Semin Neurosci* **8**:217–223.

Fagura MS, Dainty IA, McKay GD, Kirk IP, Humphries RG, Robertson MJ, Dougall IG and Leff P (1998) $P2Y_1$ receptors in human platelets which are pharmacologically distinct from $P2Y_{ADP}$ receptors. *Br J Pharmacol* **124**:157–164.

Falzoni S, Munerati M, Ferrari D, Spisani S, Moretti S and Di Virgilio F (1995) The purinergic P_{2Z} receptor of human macrophage cells: Characterization and possible physiological role. *J Clin Invest* **95**:1207–1216.

Fan Y and McCloskey MA (1994) Dual pathways for GTP-dependent regulation of chemoattractant-activated K^+ conductance in murine J774 monocytes. *J Biol Chem* **269**:31533–31543.

Fastbom J and Fredholm BB (1990) Effects of long-term theophylline treatment on adenosine A_1-receptors in rat brain: Autoradiographic evidence for increased receptor number and altered coupling to G-proteins. *Brain Res* **507**:195–199.

Fedan JS, Dagirmanjian JP, Attfield MD and Chideckel EW (1990) Evidence that the P_{2X} purinoceptor of the smooth muscle of the guinea pig vas deferens is an ATP^{4-} receptor. *J Pharmacol Exp Ther* **255**:46–51.

Fedan JS, Hogaboom GK, O'Donnell JP, Jeng SJ and Guillory RJ (1985) Interaction of [^{3}H]arylazido aminopropionyl ATP ([^{3}H]ANAPP$_3$) with P_2-purinoceptors in the smooth muscle of the isolated guinea-pig vas deferens. Eur. *J Pharmacol* **108**:49–61.

Fedan JS and Lamport SJ (1990) Effects of reactive blue 2 (RB2), p-chloromercuribenzene sulphonate (PCMBS), 4,4'-diisothiocyano-2,2'-disulphonic acid stilbene (DIDS), phorbol myristate acetate (PMA) and Cs^+ on contractions of guinea-pig isolated vas deferens (VD) to ATP. *FASEB J* **4**:A1118.

Felsch A, Stocker K and Borchard U (1995) Phorbol ester-stimulated adherence of neutrophils to endothelial cells is reduced by adenosine A_2 receptor agonists. *J Immunol* **155**:333–338.

Feoktistov I and Biaggioni I (1995) Adenosine A_{2b} receptors evoke interleukin-8 secretion in human mast cells: An enprofylline-sensitive mechanism with implications for asthma. *J Clin Invest* **96**:1979–1986.

Feoktistov I and Biaggioni I (1996) Role of adenosine in asthma. *Drug Dev Res* **39**:333–336.

Feolde E, Vigne P, Breittmayer JP and Frelin C (1995) ATP, a partial agonist of atypical P_{2Y} purinoceptors in rat brain microvascular endothelial cells. *Br J Pharmacol* **115**:1199–1203.

Ferguson DR, Kennedy I and Burton TJ (1997) ATP is released from rabbit urinary bladder by hydrostatic pressure changes- A possible sensory mechanism? *J Physiol (Lond)* **505**:503–511.

Ferrari D, Chiozzi P, Falzoni S, Dal Susino M, Melchiorri L, Baricordi OR and Di Virgilio F (1997) Extracellular ATP triggers IL-1β release by activating the purinergic P2Z receptor of human macrophages. *J Immunol* **159**:1451–1458.

Ferré S, Fredholm BB, Morelli M, Popoli P and Fuxe K (1997) Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. *Trends Neurosci* **20**:482–487.

Ferré S, Fuxe K, Von Euler G, Johansson B and Fredholm BB (1992) Adenosine-dopamine interactions in the brain. *Neuroscience* **51**:501–512.

Ferré S, Von Euler G, Johansson J, Fredholm BB and Fuxe K (1991) Stimulation of high-affinity adenosine A_2 receptors decreases the affinity of dopamine D_2 receptors in rat striatal membranes. *Proc Natl Acad Sci USA* **88**:7238–7241.

Fieber LA and Adams DJ (1991) Adenosine triphosphate-evoked currents in cultured neurons dissociated from rat parasympathetic cardiac ganglia. *J Physiol (Lond)* **434**:239–256.

Fiebich BL, Biber K, Gyufko K, Berger M, Bauer J and van Calker D (1996) Adenosine A_{2b} receptors mediate an increase in interleukin (IL)-6 mRNA and IL-6 protein synthesis in human astroglioma cells. *J Neurochem* **66**:1426–1431.

Filippov AK, Selyanko AA, Robbins J and Brown DA (1994) Activation of nucleotide receptors inhibits M-type K current [IK(M)] in neuroblastoma × glioma hybrid cells. *Pflüeg Arch Eur J Physiol* **429**:223–230.

Filippov AK, Webb TE, Barnard EA and Brown DA (1997) Inhibition by heterologously-expressed $P2Y_2$ nucleotide receptors of N-type calcium currents in rat sympathetic neurons. *Br J Pharmacol* **121**:849–851.

Filtz TM, Li Q, Boyer JL, Nicholas RA and Harden TK (1994) Expression of a cloned P_{2Y}-purinergic receptor that couples to phospholipase C. *Mol Pharmacol* **46**:8–14.

Fine J, Cole P and Davidson JS (1989) Extracellular nucleotides stimulate receptor-mediated calcium mobilization and inositol phosphate production in human fibroblasts. *Biochem J* **263**:371–376.

Fink JS, Weaver DR, Rivkees SA, Peterfreund RA, Pollack AE, Adler EM and Reppert SM (1992) Molecular cloning of the rat A_2 adenosine receptor: Selective co-expression with D_2 dopamine receptors in rat striatum. *Mol Brain Res* **14**:186–195.

Firestein BL, Xing M, Hughes RJ, Corvera CU and Insel PA (1996) Heterogeneity of P_{2U}- and P_{2Y}-purinergic receptor regulation of phospholipases in MDCK cells. *Am J Physiol* **271**:F610–F618.

Fischer B, Boyer JL, Hoyle CHV, Ziganshin AU, Brizzolara AL, Knight GE, Zimmet J, Burnstock G, Harden TK and Jacobson KA (1993) Identification of potent, selective P_{2Y}-purinoceptor agonists: Structure activity relationships for 2-thioether derivatives of adenosine 5′-triphosphate. *J Med Chem* **7**:407–409.

Fitz JG and Sostman AH (1994) Nucleotide receptors activate cation, potassium, and chloride currents in a liver cell line. *Am J Physiol* **266**:G544–G553.

Flezar M and Heisler S (1993) P2-purinergic receptors in human breast tumour cells: Coupling of intracellular calcium signaling to anion secretion. *J Pharmacol Exp Ther* **265**:1499–1510.

Flodgaard H and Klenow H (1982) Abundant amounts of the diadenosine 5′,5‴-P^1, P^4-tetraphosphate are present and releasable, but metabolically inactive in human platelets. *Biochem J* **208**:737–742.

Folkow B (1949) The vasodilator action of adenosine triphosphate. *Acta Physiol Scand* **17**:311–316.

Forrester T (1990) Release of ATP from heart: Presentation of a release model using human erythrocyte. *Ann N Y Acad Sci* **603**:335–352.

Forrester T and Lind AR (1969) Identification of adenosine triphosphate in human plasma and the concentration in the venous effluent of forearm muscles before, during and after sustained contractions. *J Physiol (Lond)* **204**:347–364.

Forsythe KM, Bjur RA and Westfall DP (1991) Nucleotide modulation of norepinephrine release from sympathetic nerves in the rat vas deferens. *J Pharmacol Exp Ther* **256**:831–826.

Fozard JR and Carruthers AM (1993) Adenosine A_3 receptors mediate hypotension in the angiotensin II-supported circulation of the pithed rat. *Br J Pharmacol* **109**:3–5.

Fozard JR and Hannon JP (1994) BW-A 522 blocks adenosine A_3 receptor-mediated hypotensive responses in the rat. *Eur J Pharmacol* **252**:R5–R6.

Fozard JR and Milavec-Krizman M (1993) Contraction of the rat isolated spleen mediated by adenosine A_1 receptor activation. *Br J Pharmacol* **109**:1059–1063.

Francis JE, Cash WD, Psychoyos S, Ghai G, Wenk P, Friedmann RC, Atkins C, Warren V, Furness P, Hyun JL, Stone GA, Desai M and Williams M (1988) Structure-activity profile of a series of novel triazoloquinazoline adenosine antagonists. *J Med Chem* **31**:1014–1020.

Fredholm BB (1982) Adenosine actions and adenosine receptors after 1 week treatment with caffeine. *Acta Physiol Scand* **115**:283–286.

Fredholm BB (1995) Purinoceptors in the nervous system. *Pharmacol Toxicol* **76**: 228–239.

Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden KT, Jacobson KA, Leff P and Williams M (1994) Nomenclature and classification of purinoceptors. *Pharmacol Rev* **46**:143–156.

Fredholm BB and Altiok N (1994) Adenosine A_{2B} receptor signalling is altered by stimulation of bradykinin or interleukin receptors in astroglioma cells. *Neurochem Int* **25**:99–102.

Fredholm BB, Burnstock G, Harden KT and Spedding M (1996) Receptor nomenclature. *Drug Dev Res* **39**:461–466.

Fredholm BB, Jonzon B and Lindgren E (1984) Changes in noradrenaline release and in beta receptor number in rat hippocampus following long-term treatment with theophylline or L-phenylisopropyladenosine. *Acta Physiol Scand* **122**:55–59.

Fredholm BB, Proctor W, van der Ploeg I and Dunwiddie TV (1989) In vivo pertussis toxin treatment attenuates some, but not all, adenosine A_1 effects in slices of the rat hippocampus. *Eur J Pharmacol* **172**:249–262.

Freissmuth M, Hausleithner V, Tuisl E, Nanoff C and Schutz W (1987) Glomeruli and microvessels of the rabbit kidney contain both A_1 and A_2-adenosine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **335**:438–444.

Freissmuth M, Schütz W and Linder ME (1991) Interactions of the bovine brain A_1-adenosine receptor with recombinant G-protein α-subunits. Selectivity for $rG_{i\alpha\text{-}3}$. *J Biol Chem* **266**:17778–17783.

Funk GD, Kanjhan R, Walsh C, Lipski J, Corner AM, Parkis MA and Housley GD (1997) P2 Receptor excitation of rodent hypoglossal motoneuron activity in vitro and in vivo: A molecular physiological analysis. *J Neurosci* **17**:6325–6337.

Furlong TJ, Pierce KD, Selbie LA and Shine J (1992) Molecular characterization of a human brain adenosine A_2 receptor. *Mol Brain Res* **15**:62–66.

Furukawa K, Ishibashi H and Akaike N (1994) ATP-induced inward current in neurons freshly dissociated from the tuberomammillary nucleus. *J Neurophysiol* **71**:868–873.

Fyffe REW and Perl ER (1984) Is ATP a central synaptic mediator for certain primary afferent fibres from mammalian skin? *Proc Natl Acad Sci USA* **81**:6890–6893.

Gaddum JH and Holtz P (1933) The localization of the action of drugs on the pulmonary vessels of dogs and cats. *J Physiol (Lond)* **77**:139–158.

Galas M-C and Harden TK (1995) Receptor-induced heterologous desensitization of receptor-regulated phospholipase C. *Eur J Pharmacol* **291**:175–182.

Galietta LJV, Rasola A, Rugolo M, Zottini M, Mastrocola T, Gruenert DC and Romeo G (1992) Extracellular 2-chloroadenosine and ATP stimulate volume-sensitive Cl^- current and calcium mobilization in human tracheal 9HTEo-cells. *FEBS Lett* **304**:61–65.

Galligan JJ, Herring A and Harpstead T (1995) Pharmacological characterization of purinoceptor-mediated constriction of submucosal arterioles in guinea pig ileum. *J Pharmacol Exp Ther* **274**:1425–1430.

Gallinaro BJ, Reimer WJ and Dixon SJ (1995) Activation of protein kinase C inhibits ATP-induced $[Ca^{2+}]_i$ elevation in rat osteoblastic cells: Selective effects on P_{2Y} and P_{2U} signaling pathways. *J Cell Physiol* **162**:305–314.

Gallo-Rodriguez C, Ji X-D, Melman N, Siegman BD, Sanders LH, Orlina J, Fischer B, Pu Q, Olah ME, Van Galen PMJ, Stiles G and Jacobson KA (1994) Structure-activity relationships of N^6-benzyladenosine-5′-uronamides as A_3-selective adenosine agonists. *J Med Chem* **37**:636–646.

Ganote CE, Armstrong S and Downey JM (1993) Adenosine and A_1 selective agonists offer minimal protection against ischaemic injury to isolated rat cardiomyocytes. *Cardiovasc Res* **27**:1670–1676.

Garcia-Guzman M, Soto F, Gomez-Hernandez JM, Lund P-E and Stühmer W (1997a) Characterization of recombinant human $P2X_4$ receptor reveals pharmacological differences to the rat homologue. *Mol Pharmacol* **51**:109–118.

Garcia-Guzman M, Soto F, Laube B and Stühmer W (1996) Molecular cloning and functional expression of a novel rat heart P_{2X} purinoceptor. *FEBS Lett* **388**:123–127.

Garcia-Guzman M, Stühmer W and Soto F (1997b) Molecular characterization and pharmacological properties of the human P_{2X3} purinoceptor. *Mol Brain Res* **47**: 59–66.

Gargett CE, Cornish EJ and Wiley JS (1996) Phospholipase D activation by P_{2Z}-purinoceptor agonists in human lymphocytes is dependent on bivalent cation influx. *Biochem J* **313**:529–535.

Gargett CE and Wiley JS (1997) The isoquinoline derivative KN-62 a potent antagonist of the P2Z-receptor of human lymphocytes. *Br J Pharmacol* **120**:1483–1490.

Garrad RC, Otero MA, Gonzalez FA, Turner JT, Clarke LL and Weisman GA (1998) Desensitization of the $P2Y_2$ receptor; implications for the therapy of cystic fibrosis. *Drug Dev Res*, **43**:12.

Garritsen A, Zhang Y and Cooper DM (1992) Purinergic receptor regulation of signal transduction in NCB-20 cells. *Mol Pharmacol* **41**:743–749.

Gerlach E, Deuticke B and Dreisbach RH (1963) Der Nucleotid-Abbau im Herzmuskel bei Sauerstoffmangel und seine mögliche Bedeutung für die Coronardurchblutung. *Naturwissenschaften* **50**:228–229.

Gerwins P and Fredholm BB (1992a) ATP and its metabolite adenosine act synergistically to mobilize intracellular calcium via the formation of inositol 1,4,5-trisphosphate in a smooth muscle cell line. *J Biol Chem* **267**:16081–16087.

Gerwins P and Fredholm BB (1992b) Stimulation of adenosine A_1-receptors and bradykinin receptors, which act via different G proteins, synergistically raised inositol 1,4,5-trisphosphate and intracellular free calcium in DDT1 MF-2 smooth muscle cells. *Proc Natl Acad Sci USA* **89**:7330–7334.

Gerwins P and Fredholm BB (1995a) Activation of adenosine A_1 and bradykinin receptors increases protein kinase C and phospholipase D activity in smooth muscle cells. *Naunyn-Schmiedeberg's Arch Pharmacol* **351**:186–193.

Gerwins P and Fredholm BB (1995b) Activation of phospholipase C and phospholipase D by stimulation of adenosine A_1, bradykinin or P_{2U} receptors does not correlate well with protein kinase C activation. *Naunyn-Schmiedeberg's Arch Pharmacol* **351**:194–201.

Gharib A, Delton I, Lagarde M and Sarda N (1992) Evidence for adenosine A_{2b} receptors in the rat pineal gland. *Eur J Pharmacol* **225**:359–360.

Gibb CA, Singh S, Cook DI, Poronnik P and Conigrave AD (1994) A nucleotide receptor that mobilizes Ca^{2+} in the mouse submandibular salivary cell line ST885. *Br J Pharmacol* **111**:1135–1139.

Gillespie JH (1934) The biological significance of the linkages in adenosine triphosphoric acid. *J Physiol (Lond)* **80**:345–349.

Gödecke S, Decking UKM, Gödecke A and Schrader J (1996) Cloning of the rat P_{2U} receptor and its potential role in coronary vasodilation. *Am J Physiol* **270**:C570–C577.

Goetz V, Da Prada M and Pletscher A (1971) Adenine-, guanine- and uridine-5′-

phosphonucleotides in blood platelets and storage organelles of various species. *J Pharmacol Exp Ther* **178**:210–215.
Gonçalves J and Queiroz G (1993) Facilitatory and inhibitory modulation by endogenous adenosine of noradrenaline release in the epididymal portion of the rat vas deferens. *Naunyn-Schmiedeberg's Arch Pharmacol* **348**:367–371.
Gonçalves J and Queiroz G (1996) Purinergic modulation of noradrenaline release in rat tail artery: Tonic modulation mediated by inhibitory P_{2Y}- and facilitatory A_{2A}-purinoceptors. *Br J Pharmacol* **117**:156–160.
Gonzalez FA, Ahmed AH, Lustig KD, Erb L and Weisman GA (1989a) Permeabilization of transformed mouse fibroblasts by 3'-O-(4-benzoyl adenosine 5'-triphosphate and the desensitization of the process. *J Cell Physiol* **139**:109–115.
Gonzalez FA, Bonapace E, Belzer I, Friedberg I and Heppel LA (1989b) Two distinct receptors for ATP can be distinguished in Swiss 3T6 mouse fibroblasts by their desensitization. *Biochem Biophys Res Commun* **164**:706–713.
Gonzalez FA, Rozengurt E and Heppel LA (1989c) Extracellular ATP induces the release of calcium from intracellular stores without the activation of protein kinase C in Swiss 3T6 mouse fibroblasts. *Proc Natl Acad Sci USA* **86**:4530–4534.
Gordon JL (1986) Extracellular ATP: Effects, sources and fate. *Biochem J* **233**:309–319.
Green A (1987) Adenosine receptor down-regulation and insulin resistance following prolonged incubation of adipocytes with an A_1 adenosine receptor agonist. *J Biol Chem* **262**:15702–15707.
Green A, Milligan G and Dobias SB (1992) Gi down-regulation as a mechanism for heterologous desensitization in adipocytes. *J Biol Chem* **267**:3223–3229.
Green AC, Dowdall MJ and Richardson CM (1997) ATP acting on P_{2Y} receptors triggers calcium mobilization in Schwann cells at the neuroelectrocyte junction in skate. *Neuroscience* **80**:635–651.
Green HN and Stoner HB (1950) *Biological Actions of Adenine Nucleotides.* H. K. Lewis & Co. Ltd., London.
Green RM and Stiles GL (1986) Chronic caffeine ingestion sensitizes the A_1 adenosine receptor-adenylate cyclase system in rat cerebral cortex. *J Clin Invest* **77**: 222–227.
Greenberg S, Di Virgilio F, Steinberg TH and Silverstein SC (1988) Extracellular nucleotides mediate Ca^{2+} fluxes in J774 macrophages by two distinct mechanisms. *J Biol Chem* **263**:10337–10343.
Greene RW and Haas HL (1991) The electrophysiology of adenosine in the mammalian nervous system. *Prog Neurobiol* **36**:329–341.
Grierson JP and Meldolesi J (1995a) Calcium homeostasis in mouse fibroblast cells: Affected by U-73122, a putative phospholiase Cβ blocker, via multiple mechanisms. *Br J Pharmacol* **115**:11–14.
Grierson JP and Meldolesi J (1995b) Shear stress-induced $[Ca^{2+}]_i$ transients and oscillations in mouse fibroblasts are mediated by endogenously released ATP. *J Biol Chem* **270**:4451–4456.
Griffiths RJ, Stam EJ, Downs JT and Otterness IG (1995) ATP induces the release of IL-1 from LPS-primed cells in vivo. *J Immunol* **154**:2821–2828.
Grover GJ, Sleph PG and Dzwonczyk S (1992) Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the dog heart and their possible interaction with adenosine A_1-receptors. *Circulation* **86**:1310–1326.
Gu J and MacDermott AB (1997) Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. *Nature (Lond.)* **389**:749–753.
Gubitz AK, Widdowson L, Kurokawa M, Kirkpatrick KA and Richardson PJ (1996) Dual signalling by the adenosine A_{2a} receptor involves activation of both N- and P-type calcium channels by different G proteins and protein kinases in the same nerve terminals. *J Neurochem* **67**:374–381.
Guibert C, Pacaud P, Loirand G, Marthan R and Savineau JP (1996) Effect of extracellular ATP on cytosolic Ca^{2+} concentration in rat pulmonary artery myocytes. *Am J Physiol* **271**:L450–L458.
Gurden MF, Coates J, Ellis F, Evans B, Foster M, Hornby E, Kennedy I, Martin DP, Strong P, Vardey CJ and Wheeldon A (1993) Functional characterization of three adenosine receptor types. *Br J Pharmacol* **109**:693–698.
Gustafsson LE, Wiklund CU, Wiklund NP and Stelius L (1990) Subclassification of neuronal adenosine receptors, in *Purines in Cellular Signaling: Targets for New Drugs* (Jacobson KA, Daly JW and Manganiello V) pp 200–205, Springer-Verlag, New York.
Häggblad J and Fredholm BB (1987) Adenosine and neuropeptide Y enhance α_1-adrenoceptor-induced accumulation of inositol phosphates and attenuate forskolin-induced accumulation of cyclic AMP in rat vas deferens. *Neurosci Lett* **82**:211–216.
Hall DA and Hourani SM (1993) Effects of analogs of adenine nucleotides on increases in intracellular calcium mediated by P_{2T}-purinoceptors on human blood platelets. *Br J Pharmacol* **108**:728–733.
Hallam TJ and Rink TJ (1985) Responses to adenosine diphosphate in human platelets loaded with the fluorescent calcium indicator quin2. *J Physiol (Lond)* **368**:131–146.
Hancock DL and Coupar IM (1995a) Functional characterization of the adenosine receptor mediating inhibition of intestinal secretion. *Br J Pharmacol* **114**:152–156.
Hancock DL and Coupar IM (1995b) Functional characterization of the adenosine receptor mediating inhibition of peristalsis in the rat jejunum. *Br J Pharmacol* **115**:739–744.
Hannon JP, Pfannkuche HJ and Fozard JR (1995) A role for mast cells in adenosine A_3 receptor-mediated hypotension in the rat. *Br J Pharmacol* **115**:945–952.
Hansmann G, Bültmann R, Tuluc F and Starke K (1997) Characterization by antagonists of P2-receptors mediating endothelium-dependent relaxation in the rat aorta. *Naunyn-Schmiedeberg's Arch Pharmacol* **356**:641–652.
Harden TK, Boyer JL and Nicholas RA (1995) P_2-Purinergic receptors: Subtype-associated signaling responses and structure. *Ann Rev Pharmacol Toxicol* **35**:541–579.
Hargreaves MB, Stoggall SM and Collis MG (1991) Evidence that the adenosine receptor mediating relaxation in dog lateral saphenous vein and guinea-pig aorta is of the A_{2b} subtype. *Br J Pharmacol* **102**:198P.
Harms L, Finta EP, Tschöpl M and Illes P (1992) Depolarization of rat locus coeruleus neurons by adenosine 5'-triphosphate. *Neuroscience* **48**:941–952.
Harrison MJ, Brossmer R and Goody RS (1975) Inhibition of platelet aggregation and the platelet release reaction by α,ω-diadenosine polyphosphates. *FEBS Lett* **54**:57–60.
Hashimoto M and Kokubun S (1995) Contribution of P_2-purinoceptors to neurogenic contraction of rat urinary bladder smooth muscle. *Br J Pharmacol* **115**:636–640.
Hashimoto K, Kumakura S and Tanemura I (1964) Mode of action of adenine, uridine and cytidine nucleotides and 2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido-(5,4-d) pyrimidine on the coronary, renal and femoral arteries. *Arzneim-Forsch* **14**:1252–1254.
Hasuo H, Shoji S, Gallagher JP and Akasu T (1992) Adenosine inhibits the synaptic potentials in rat septal neurons mediated through pre- and postsynaptic A_1-adenosine receptors. *Neurosci Res* **13**:281–299.
Hauber W and Munkle M (1995) Stimulation of adenosine A_{2a} receptors in the rat striatum induces catalepsy that is reversed by antagonists of N-methyl-D-aspartate receptors. *Neurosci Lett* **196**:205–208.
Haussinger D, Stehle T and Gerok W (1987) Actions of extracellular UTP and ATP in perfused rat liver: A comparative study. *Eur J Biochem* **167**:65–71.
Hawkins M, Dugish MM, Porter NM, Urbancic M and Radulovacki M (1988) Effects of chronic administration of caffeine on adenosine A_1 and A_2 receptors in rat brain. *Brain Res Bull* **21**:479–482.
Haynes JJR, Obiako B, Thompson WJ and Downey J (1995) Adenosine-induced vasodilation: Receptor characterization in pulmonary circulation. *Am J Physiol* **268**:H1862–H1868.
Hechler B, Eckly A, Ohlmann P, Cazenave JP and Gachet C (1998) The $P2Y_1$ receptor is necessary but not sufficient to support full ADP-induced platelet aggregation: Evidence for the presence of another P2Y receptor. *Drug Dev Res,* **43**:15.
Henderson DJ, Elliot DG, Smith GM, Webb TE and Dainty IA (1995) Cloning and characterisation of a bovine P_{2Y} receptor. *Biochem Biophys Res Commun* **212**:648–656.
Hendrikx M, Toshima Y, Mubagwa K and Flameng W (1993) Improved functional recovery after ischemic preconditioning in the globally ischemic rabbit heart is not mediated by adenosine A_1 receptor activation. *Basic Res Cardiol* **88**:576–593.
Henning RH, Duin M, Den Hertog A and Nelemans A (1993) Activation of the phospholipase C pathway by ATP is mediated exclusively through nucleotide type P_2-purinoceptors in C2C12 myotubes. *Br J Pharmacol* **110**:747–752.
Henning RH, Nelemans A, van den Akker J and Den Hertog A (1992) The nucleotide receptors on mouse C2C12 myotubes. *Br J Pharmacol* **106**:853–858.
Herold CL, Li Q, Schachter JB, Harden TK and Nicholas RA (1997) Lack of nucleotide-promoted second messenger signaling responses in 1321N1 cells expressing the proposed P2Y receptor, p2y7. *Biochem Biophys Res Commun* **235**:717–721.
Heurteaux C, Lauritzen I, Widmann C and Lazdunski M (1995) Essential role of adenosine, adenosine A_1 receptors, and ATP-sensitive K^+ channels in cerebral ischemic preconditioning. *Proc Natl Acad Sci USA* **92**:4666–4670.
Hickman SE, El-Khoury J, Greenberg S, Schieren I and Siverstein SC (1994) P_{2Z} adenosine triphosphate receptor activity in cultured human monocyte-derived macrophages. *Blood* **84**:2452–2456.
Hide I, Padgett WL, Jacobson KA and Daly JW (1992) A_{2A} adenosine receptors from rat striatum and rat pheochromocytoma PC12 cells: Characterization with radioligand binding and by activation of adenylate cyclase. *Mol Pharmacol* **41**:352–359.
Hiley CR, Bottrill FE, Warnock J and Richardson PJ (1995) Effects of pH on responses to adenosine, CGS 21680, carbachol and nitroprusside in the isolated perfused superior mesenteric arterial bed of the rat. *Br J Pharmacol* **116**:2641–2646.
Hill RJ, Oleynek JJ, Hoth CF, Kiron MAR, Weng W, Wester RT, Tracey R, Knight DR, Buchholz RA and Kennedy SP (1997) Cloning, expression and pharmacological characterization of rabbit adenosine A_1 and A_3 receptors. *J Pharmacol Exp Ther* **280**:122–128.
Hillaire-Buys D, Bertrand G, Chapal J, Puech R, Ribes G and Loubatières-Mariani MM (1993) Stimulation of insulin secretion and improvement of glucose tolerance in rat and dog by the P_{2y}-purinoceptor agonist, adenosine-5'-O-((2-thiodiphosphate). *Br J Pharmacol* **109**:183–187.
Hillaire-Buys D, Chapal J, Bertrand G, Petit P and Loubatières-Mariani MM (1994) Purinergic receptors on insulin-secreting cells. *Fundam Clin Pharmacol* **8**:117–127.
Hillaire-Buys D, Chapal J, Petit P and Loubatières-Mariani MM (1991) Dual regulation of pancreatic vascular tone by P_{2X} and P_{2Y} purinoceptor subtypes. *Eur J Pharmacol* **199**:309–314.
Hillaire-Buys D, Gross R, Loubatières-Mariani MM and Ribes G (1989) Effect of pertussis toxin on A_1-receptor-mediated inhibition of insulin secretion. *Br J Pharmacol* **96**:3–4.
Hindley S, Herman MAR and Rathbone MP (1994) Stimulation of reactive astrogliosis in vivo by extracellular adenosine diphosphate or an A_2 receptor agonist. *J Neurosci Res* **38**:399–406.
Hiruma H and Bourque CW (1995) P_2 purinoceptor-mediated depolarization of rat supraoptic neurosecretory cells in vitro. *J Physiol (Lond)* **489**:3: 805–811.
Ho C, Hicks J and Salter MW (1995) A novel P_2-purinoceptor expressed by a subpopulation of astrocytes from the dorsal spinal cord of the rat. *Br J Pharmacol* **116**:2909–2918.
Hogaboom GK, O'Donnell JP and Fedan JS (1980) Purinergic receptors: Photoaffinity analog of adenosine triphosphate is a specific adenosine triphosphate antagonist. *Science (Wash. DC)* **208**:1273–1276.
Hoiting B, Molleman A, Nelemans A and Den Hertog A (1990) P_2-purinoceptor-activated membrane currents and inositol tetrakisphosphate formation are blocked by suramin. *Eur J Pharmacol* **181**:127–131.
Holton FA and Holton P (1953) The possibility that ATP is a transmitter at sensory nerve endings. *J Physiol (Lond)* **119**:50–51P.
Holton P (1959) The liberation of ATP on antidromic stimulation of sensory nerves. *J Physiol (Lond)* **145**:494–504.

Honoré H, Martin C, Mironneau C and Mironneau J (1989) An ATP-sensitive conductance in cultured smooth muscle cells from pregnant rat myometrium. *Am J Physiol* **257**:C294–C305.

Hopwood AM and Burnstock G (1987) ATP mediates coronary constriction via P_{2X}-purinoceptors and coronary vasodilatation via P_{2Y}-purinoceptors in the isolated perfused rat heart. *Eur J Pharmacol* **136**:49–54.

Hourani SMO, Bailey SJ, Nicholls J and Kitchen I (1991) Direct effects of adenylyl 5′-(β,γ-methylene)diphosphonate, a stable ATP analog, on relaxant P_1-purinoceptors in smooth muscle. *Br J Pharmacol* **104**:685–690.

Hourani SMO and Hall DA (1996) P2T purinoceptors: ADP receptors on platelets, in *P2 Purinoceptors: Localization, Function and Transduction Mechanisms* (Chadwick DJ and Goode JA eds) pp 53–70, John Wiley & Sons, Chichester.

Hourani SMO, Hall DA and Nieman CJ (1992) Effects of the P_2-purinoceptor antagonist suramin on human platelet aggregation induced by adenosine 5′-diphosphate. *Br J Pharmacol* **105**:453–457.

Hourani SMO, Johnson CR and Bailey SJ (1993) Desensitization of the P_2-purinoceptors on the rat colon muscularis mucosae. *Br J Pharmacol* **110**:501–505.

Hourani SMO and Jones DA (1994) Post-junctional excitatory adenosine A_1 receptors in the rat vas deferens. *Gen Pharmacol* **25**:417–420.

Housley GD, Greenwood D, Bennett T and Ryan AF (1995) Identification of a short form of the P_{2xR1}-purinoceptor subunit produced by alternative splicing in the pituitary and cochlea. *Biochem Biophys Res Commun* **212**:501–508.

Houston DA, Burnstock G and Vanhoutte PM (1987) Different P_2-purinergic receptor subtypes of the endothelium and smooth muscle in canine blood vessels. *J Pharmacol Exp Ther* **241**:501–506.

Hoyle CHV (1990) Pharmacological activity of adenine dinucleotides in the periphery: Possible receptor classes and transmitter function. *Gen Pharmacol* **21**:827–831.

Hoyle CHV, Chapple C and Burnstock G (1989) Isolated human bladder: Evidence for an adenine dinucleotide acting on P_{2X}-purinoceptors and for purinergic transmission. *Eur J Pharmacol* **174**:115–118.

Hoyle CH, Knight GE and Burnstock G (1990) Suramin antagonizes responses to P_2-purinoceptor agonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia coli. *Br J Pharmacol* **99**:617–621.

Hoyle CHV, Ziganshin AU, Pintor J and Burnstock G (1996) The activation of P_1- and P_2-purinoceptors in the guinea-pig left atrium by diadenosine polyphosphates. *Br J Pharmacol* **118**:1294–1300.

Hu H-Z and Li Z-W (1997) Modulation by adenosine of GABA-activated current in rat dorsal root ganglion neurons. *J Physiol (Lond)* **501**:67–75.

Humphrey PPA, Buell G, Kennedy I, Khakh BS, Michel AD, Surprenant A and Trezise DJ (1995) New insights on P_{2X} purinoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **352**:585–596.

Humphreys BD and Dubyak GR (1996) Induction of the P_{2Z}/$P2X_7$ nucleotide receptor and associated phospholipase D activity by lipopolysaccharide and IFN-gamma in the human THP-1 monocytic cell line. *J Immunol* **157**:5627–5637.

Humphries RG, Tomlinson W, Clegg JA, Ingall AH, Kindon ND and Leff P (1995) Pharmacological profile of the novel P_{2T}-purinoceptor antagonist, FPL 67085 in vitro and in the anaesthetized rat in vivo. *Br J Pharmacol* **115**:1110–1116.

Humphries RG, Tomlinson W, Ingall AH, Cage PA and Leff P (1994) FPL 66096: A novel, highly potent and selective antagonist at human platelet P_{2T}-purinoceptors. *Br J Pharmacol* **113**:1057–1063.

Hutchison AJ, Williams M, de Jesus R, Yokoyama R, Oei HH, Ghai GR, Webb RL, Zoganas HC, Stone GA and Jarvis MF (1990) 2-(Arylalkylamino)adenosine-5′-uronamides: A new class of highly selective adenosine A_2 receptor ligands. *J Med Chem* **33**:1919–1924.

Huttemann E, Ukena D, Lenschow V and Schwabe U (1984) R_a adenosine receptors in human platelets: Characterization by 5′-*N*-ethylcarboxamido[^{3}H]-adenosine binding in relation to adenylate cyclase activity. *Naunyn Schmiedeberg's Arch Pharmacol* **325**:226–233.

Huwiler A and Pfeilschifter J (1994) Stimulation of extracellular ATP and UTP of the mitogen-activated protein kinase cascade and proliferation of rat renal mesangial cells. *Br J Pharmacol* **113**:1455–1463.

Huwiler A, van Rossum G, Wartmann M and Pfeilschifter J (1997) Stimulation by extracellular ATP and UTP of the stress-activated protein kinase cascade in rat renal mesangial cells. *Br J Pharmacol* **120**:807–812.

Igusa Y (1988) Adenosine 5′-triphosphate activates acetylcholine receptor channels in cultured *Xenopus* myotomal muscle cells. *J Physiol (Lond)* **405**:169–185.

Ijzerman AP, von Frijtag Drabbe Künzel JK, Kim J, Jiang Q and Jacobson KA (1996) Site-directed mutagenesis of the human A_{2A} receptor: Critical involvement of Glu^{13} in agonist recognition. *Eur J Pharmacol* **310**:269–272.

Ikeda M, Koizumi S, Nakazawa K, Inoue K, Ito K and Inoue K (1996) Potentiation by cadmium ion of ATP-evoked dopamine release in rat phaeochromocytoma cells. *Br J Pharmacol* **117**:950–954.

Ikeda K, Suzuki M, Furukawa M and Takasaka T (1995) Calcium mobilization and entry induced by extracellular ATP in the non-sensory epithelial cell of the cochlear lateral wall. *Cell Calcium* **18**:89–99.

Ikeuchi Y and Nishizaki T (1995a) ATP-evoked potassium currents in rat striatal neurons are mediated by a P_2 purinergic receptor. *Neurosci Lett* **190**:89–92.

Ikeuchi Y and Nishizaki T (1995b) The P_{2Y} purinoceptor-operated potassium channel is possibly regulated by the βγ subunits of a pertussis toxin-insensitive G-protein in cultured rat inferior colliculus neurons. *Biochem Biophys Res Commun* **214**:589–596.

Ikeuchi Y and Nishizaki T (1996a) P_2-purinoceptor-operated potassium channel in rat cerebellar neurons. *Biochem Biophys Res Commun* **218**:67–71.

Ikeuchi Y and Nishizaki T (1996b) ATP-regulated K^+ channel and cytosolic Ca^{2+} mobilization in cultured rat spinal neurons. *Eur J Pharmacol* **302**:163–169.

Ikeuchi Y, Nishizaki T, Mori M and Okada Y (1995b) Adenosine activates the potassium channel via a P_2 purinoceptor but not via an adenosine receptor in cultured rat superior colliculus neurons. *Neurosci Lett* **198**:205–208.

Ikeuchi Y, Nishizaki T, Mori M and Okada Y (1996a) Adenosine activates the K^+ channel and enhances cytosolic Ca^{2+} release via a P_{2Y} purinoceptor in hippocampal neurons. *Eur J Pharmacol* **304**:191–199.

Ikeuchi Y, Nishizaki T, Mori M and Okada Y (1996b) Regulation of the potassium current and cytosolic Ca^{2+} release induced by 2-methylthio ATP in hippocampal neurons. *Biochem Biophys Res Commun* **218**:428–433.

Ikeuchi Y, Nishizaki T and Okada Y (1995a) A P_2 purinoceptor activated by ADP in rat medullar neurons. *Neurosci Lett* **198**:71–74.

Inoue CN, Woo JS, Schwiebert EM, Morita T, Hanaoka K, Guggino SE and Guggino WB (1997) Role of purinergic receptors in chloride secretion in Caco-2 cells. *Am J Physiol* **272**:C1862–C1870.

Inoue K, Nakazawa K, Fujimori K, Watano T and Takanaka A (1992) Extracellular adenosine 5′-triphosphate-evoked glutamate release in cultured hippocampal neurons. *Neurosci Lett* **134**:215–218.

Inoue K, Nakazawa K, Ohara-Imaizumi M, Obama T, Fujimori K and Tanaka A (1991) Selective and competitive antagonism by suramin of ATP-stimulated catecholamine-secretion from PC 12 phaeochromocytoma cells. *Br J Pharmacol* **102**: 581–584.

Iredale PA, Alexander SPH and Hill SJ (1994) Coupling of a transfected human brain A1 adenosine receptor in CHO-K1 cells to calcium mobilisation via a pertussis toxin-sensitive mechanism. *Br J Pharmacol* **111**:1252–1256.

Iredale PA and Hill SJ (1993) Increases in intracellular calcium via activation of an endogenous P_2-purinoceptor in cultured CHO-K1 cells. *Br J Pharmacol* **110**:1305–1310.

Ishii R, Shinozuka K, Kunitomo M, Hashimoto T and Takeuchi K (1995) Characterization of the facilitatory prejunctional purinoceptor on adrenergic nerves of the rabbit ear artery. *J Pharmacol Exp Ther* **273**:1390–1395.

Ishikawa S, Saijoh K and Okada Y (1997) Endogenous adenosine facilitates neurotransmission via A_{2A} adenosine receptors in the rat superior colliculus in vivo. *Brain Res* **757**:268–275.

Ito H, Hosoya Y, Inanobe A, Tomoike H and Endoh M (1995) Acetylcholine and adenosine activate the G protein-gated muscarinic K^+ channel in ferret ventricular myocytes. *Naunyn Schmiedeberg's Arch Pharmacol* **351**:610–617.

Ito H, Vereecke J and Carmeliet E (1994) Mode of regulation by G protein of the ATP-sensitive K^+ channel in guinea-pig ventricular cell membrane. *J Physiol (Lond)* **478**:101–108.

Iwamoto T, Umemura S, Toya Y, Uchibori T, Kogi K, Takagi N and Ishii M (1994) Identification of adenosine A_2 receptor-cAMP system in human aortic endothelial cells. *Biochem Biophys Res Commun* **199**:905–910.

Jabs R, Paterson IA and Walz W (1997) Qualitative analysis of membrane currents in glial cells from normal and gliotic tissue in situ: Down-regulation of Na^+ current and lack of P_2 purinergic responses. *Neuroscience* **81**:847–860.

Jacobson KA, Nikodijevic O, Padgett WL, Gallo-Rodriguez C, Maillard M and Daly JW (1993a) 8-(3-Chlorostyryl)caffeine (CSC) is a selective A_2-adenosine antagonist in vitro and in vivo. *FEBS Lett* **323**:141–144.

Jacobson KA, Nikodijevic O, Shi D, Gallo-Rodriguez C, Olah ME, Stiles GL and Daly JW (1993b) A role for central A_3-adenosine receptors. Mediation of behavioural depressant effects. *FEBS Lett* **336**:57–60.

Jacobson KA, Park K-S, Jiang J-L, Kim Y-C, Olah ME, Stiles GL and Ji X-D (1997) Pharmacological characterization of novel A_3 adenosine receptor-selective antagonists. *Neuropharmacology* **36**:1157–1165.

Jacobson KA, Stiles GL and Ji XD (1992a) Chemical modification and irreversible inhibition of striatal A_{2a} adenosine receptors. *Mol Pharmacol* **42**:123–133.

Jacobson KA, van Galen PJM, Ji X-O, Ramkumar V, Olah M and Stiles M (1993c) Molecular characterization of A_1 and A_{2a} receptors. *Drug Dev Res* **28**:226–231.

Jacobson KA, van Galen PJM and Williams M (1992b) Adenosine receptors: Pharmacology, structure-activity relationships, and therapeutic potential. *J Med Chem* **35**:407–422.

Jacobson MA, Chakravarty PK, Johnson RG and Norton R (1996) Novel selective non-xanthine A_3 adenosine receptor antagonists. *Drug Dev Res* **37**:131.

Jacobson MA, Johnson RG, Luneau CJ and Salvatore CA (1995a) Cloning and chromosomal localization of the human A_{2b} adenosine receptor gene (ADORA2B) and its pseudogene. *Genomics* **27**:374–376.

Jahr CE and Jessel TM (1983) ATP excites a subpopulation of rat dorsal horn neurons. *Nature (Lond.)* **304**:730–733.

Jain N, Kemp N, Adeyemo O, Buchanan P and Stone TW (1995) Anxiolytic activity of adenosine receptor activation in mice. *Br J Pharmacol* **116**:2127–2133.

Jamieson GP, Snook MB, Thurlow PJ and Wiley JS (1996) Extracellular ATP causes loss of L-selectin from human lymphocytes via occupancy of P_{2Z} purinoceptors. *J Cell Physiol* **166**:637–642.

Janssens R, Communi D, Pirotton S, Samaon M, Parmentier M and Boeynaems J-M (1996) Cloning and tissue distribution of the human $P2Y_1$ receptor. *Biochem Biophys Res Commun* **221**:588–593.

Jarvis MF, Schulz R, Hutchison AL, Do UH, Sillis MA and Williams M (1989) [^{3}H]CGS 21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. *J Pharmacol Exp Ther* **251**:888–893.

Ji X-D, Von Lubitz D, Olah ME, Stiles GL and Jacobson KA (1994) Species differences in ligand affinity at central A_3-adenosine receptors. *Drug Dev Res* **33**:51–59.

Jiang Q, Guo D, Lee BX, Van Rhee AM, Kim Y-C, Nicholas RA, Schachter JB, Harden TK and Jacobson KA (1997) A mutational analysis of residues essential for ligand recognition at the human $P2Y_1$ receptor. *Mol Pharmacol* **52**:499–507.

Jin J, Daniel JL and Kunapuli SP (1998) Molecular basis for ADP-induced platelet activation: II. The $P2Y_1$ receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. *J Biol Chem* **273**:2030–2034.

Jin S and Fredholm BB (1997) Adenosine A_{2A} receptor stimulation increases release of acetylcholine from rat hippocampus but not striatum, and does not affect catecholamine release. *Naunyn-Schmiedeberg's Arch Pharmacol* **355**:48–56.

Jockers R, Linder ME, Hohenegger M, Nanoff C, Bertin B, Strosberg AD, Marullo S and Freissmuth M (1994) Species difference in the G protein selectivity of the human and bovine A_1-adenosine receptor. *J Biol Chem* **269**:32077–32084.

Johansson B, Ahlberg S, van Der Ploeg I, Brené S, Lindefors N, Persson H and Fredholm BB (1993a) Effect of long term caffeine treatment on A_1 and A_2 adeno-

sine receptor binding and on mRNA levels in rat brain. *Naunyn-Schmiedeberg's Arch Pharmacol* **347**:407–414.
Johansson B, Georgiev V, Parkinson FE and Fredholm BB (1993b) The binding of the adenosine A_2 receptor selective agonist [^{3}H]CGS 21680 to rat cortex differs from its binding to rat striatum. *Eur J Pharmacol* **247**:103–110.
Johnson CR, Charlton SJ and Hourani SMO (1996) Responses of the longitudinal muscle and the muscularis mucosae of the rat duodenum to adenine and uracil nucleotides. *Br J Pharmacol* **117**:823–830.
Juul B, Plesner L and Aalkjaer C (1992) Effects of ATP and UTP on $[Ca^{2+}]_i$, membrane potential and force in isolated rat small arteries. *J Vasc Res* **29**:385–395.
Kafka SH and Corbett R (1996) Selective adenosine A_{2A} receptor/dopamine D_2 receptor interactions in animal models of schizophrenia. *Eur J Pharmacol* **295**: 147–154.
Kamada S, Blackmore PF, Oehninger S, Gordon K and Hodgen GD (1994) Existence of P_2-purinoceptors on human and porcine granulosa cells. *J Clin Endocrinol Metab* **78**:650–656.
Kanda T, Shiozaki S, Shimada J, Suzuki F and Nakamura J (1994) KF17837: A novel selective adenosine A_{2A} receptor antagonist with anticataleptic activity. *Eur J Pharmacol* **256**:263–268.
Kaplan AD, Kilkenny DM, Hill DJ and Dixon SJ (1996) Extracellular nucleotides act through P_{2U} purinoceptors to elevate $[Ca^{2+}]_i$ and enhance basic fibroblast growth factor-induced proliferation in sheep chondrocytes. *Endocrinology* **137**:4757–4766.
Karlsten R, Gordh T and Post C (1992) Local antinociceptive and hyperalgesic effects in the formalin test after peripheral administration of adenosine analogs in mice. *Pharmacol Toxicol* **70**:434–438.
Kawazoe K, Matsumato M, Tanabe S, Fujiwara M, Yanagimoto M, Hirata M and Kakiuchi K (1980) Coronary and cardiohemodynamic effects of 2-phenylaminoadenosine (CV 1808) in anaesthetized dogs and cats. *Arzneim-Forsch* **30**: 1083–1087.
Keefe KD, Pasco JS and Eckman DM (1992) Purinergic relaxation and hyperpolarization in guinea pig and rabbit coronary artery: Role of the endothelium. *J Pharmacol Exp Ther* **260**:592–600.
Kennedy C and Burnstock G (1985) Evidence for two types of P_2-purinoceptor in the longitudinal muscle of the rabbit portal vein. *Eur J Pharmacol* **111**:49–56.
Kennedy C, Delbro D and Burnstock G (1985) P_2-purinoceptors mediate both vasodilation (via the endothelium) and vasoconstriction of the isolated rat femoral artery. *Eur J Pharmacol* **107**:161–168.
Kennedy I and Humphrey PP (1994) Evidence for the presence of two types of P_2 purinoceptor in the guinea-pig ileal longitudinal smooth muscle preparation. *Eur J Pharmacol* **261**:273–280.
Keppens S and De Wulf H (1991) Characterization of the biological effects of 2-methylthio-ATP on rat hepatocytes: Clear-cut differences with ATP. *Br J Pharmacol* **104**:301–304.
Keppens S, Vandekerckhove A and De Wulf H (1992) Extracellular ATP and UTP exert similar effects on rat isolated hepatocytes. *Br J Pharmacol* **105**:475–492.
Khakh BS, Humphrey PPA and Henderson G (1997) ATP-gated cation channels (P_{2X} purinoceptors) in trigeminal mesencephalic nucleus neurons of the rat. *J Physiol (Lond)* **498**:709–715.
Khakh BS, Humphrey PP and Surprenant A (1995a) Electrophysiological properties of P_{2X}-purinoceptors in rat superior cervical, nodose and guinea-pig coeliac neurons. *J Physiol (Lond)* **484**:385–395.
Khakh BS, Michel A and Humphrey PPA (1994) Estimates of antagonist affinities at P_{2X} purinoceptors in rat vas deferens. *Eur J Pharmacol* **263**:301–309.
Khakh BS, Surprenant A and Humphrey PPA (1995b) A study on P_{2X} purinoceptors mediating the electrophysiological and contractile effects of purine nucleotides in rat vas deferens. *Br J Pharmacol* **115**:177–185.
Kidd EJ, Grahames BA, Simon J, Michel AD, Barnard EA and Humphrey PPA (1995) Localization of P_{2X} purinoceptor transcripts in the rat nervous system. *Mol Pharmacol* **48**:569–573.
Kim HO, Ji XD, Siddiqi SM, Olah ME, Stiles GL and Jacobson KA (1994) 2-Substitution of N^6-benzyladenosine-5'-uronamides enhances selectivity for A_3 adenosine receptors. *J Med Chem* **37**:3614–3621.
Kim KC, Park HR, Shin CY, Akiyama T and Ko KH (1996) Nucleotide-induced mucin release from primary hamster tracheal surface epithelial cells involves the P_{2U} purinoceptor. *Eur Respir J* **9**:542–548.
Kim WK and Rabin RA (1994) Characterization of the purinergic P_2 receptors in PC12 cells: Evidence for a novel subtype. *J Biol Chem* **269**:6471–6477.
Kim J, Wess J, van-Rhee AM, Schoneberg T and Jacobson KA (1995) Site-directed mutagenesis identifies residues involved in ligand recognition in the human A_{2a} adenosine receptor. *J Biol Chem* **270**:13987–13997.
Kimball BC and Mulholland MW (1996) Enteric glia exhibit P_{2U} receptors that increase cytosolic calcium by a phospholipase C-dependent mechanism. *J Neurochem* **66**:604–612.
Kimelberg HK, Cai Z, Rastogi P, Charniga CJ, Goderie S, Dave V and Jalonen TO (1997) Transmitter-induced calcium responses differ in astrocytes acutely isolated from rat brain and in culture. *J Neurochem* **68**:1088–1098.
King BF, Chen C-C, Akopian AN, Burnstock G and Wood JN (1997) A role for calcineurin in the desensitization of the $P2X_3$ receptor. *Neuroreport* **8**:1099–1102.
King BF, Pintor J, Wang S, Ziganshin AU, Ziganshina LE and Burnstock G (1996a) A novel P_1 purinoceptor activates an outward K^+ current in follicular oocytes of *Xenopus laevis*. *J Pharmacol Exp Ther* **276**:93–100.
King BF, Ziganshina LE, Pintor J and Burnstock G (1996b) Full sensitivity of P_{2X2} purinoceptor to ATP revealed by changing extracellular pH. *Br J Pharmacol* **117**:1371–1373.
Kirischuk S, Moller T, Voitenko N, Kettenmann H and Verkhratsky A (1995b) ATP-induced cytoplasmic calcium mobilization in Bergmann glial cells. *J Neurosci* **15**:7861–7871.
Kirischuk S, Scherer J, Kettenmann H and Verkhratsky A (1995a) Activation of P_2-purinoceptors triggered Ca^{2+} release from $InsP_3$-sensitive internal stores in mammalian oligodendrocytes. *J Physiol (Lond)* **483**:41–57.
Kirk IP and Richardson PJ (1995) Inhibition of striatal GABA release by the adenosine A_{2a} receptor is not mediated by increases in cyclic AMP. *J Neurochem* **64**:2801–2809.
Kirkpatrick KA and Richardson PJ (1993) Adenosine receptor-mediated modulation of acetylcholine release from rat striatal synaptosomes. *Br J Pharmacol* **110**:949–954.
Kirsch GE, Codina J, Birnbaumer L and Brown AM (1990) Coupling of ATP-sensitive K^+ channels to A_1 receptors by G proteins in rat ventricular myocytes. *Am J Physiol* **259**:H820–H826.
Kleppisch T and Nelson MT (1995) Adenosine activates ATP-sensitive potassium channels in arterial myocytes via A_2 receptors and cAMP-dependent protein kinase. *Proc Natl Acad Sci USA* **92**:12441–12445.
Klishin A, Lozovaya N, Pintor J, Miras-Portugal MT and Krishtal OA (1994) Possible functional role of diadenosine polyphosphates: Negative feedback for excitation in the hippocampus. *Neuroscience* **58**:235–236.
Klotz KN, Hessling J, Hegler J, Owman C, Kull B, Fredholm BB and Lohse MJ (1998) Comparative pharmacology of human adenosine receptor subtypes: Characterization of stably transfected receptors in CHO cells. *Naunyn-Schmiedeberg's Arch Pharmacol* **357**:1–9.
Knight GE and Burnstock G (1993) Identification of purinoceptors in the isolated stomach and intestine of the three-spined stickleback *Gasterosteus aculeatus L. Comp Biochem Physiol C* **106**:71–78.
Knight GE and Burnstock G (1996) The effects of purine compounds on the isolated aorta of the frog *Rana temporaria*. *Br J Pharmacol* **117**:873–878.
Ko WH, O'Dowd JJ, Pediani JD, Bovell DL, Elder HY, Jenkinson DM and Wilson SM (1994) Extracellular ATP can activate autonomic signal transduction pathways in cultured equine sweat gland epithelial cells. *J Exp Biol* **190**:239–252.
Kohno Y, Ji X-D, Mawhorter SD, Koshiba M and Jacobson KA (1996a) Activation of A_3 adenosine receptors on human eosinophils elevates intracellular calcium. *Blood* **88**:3569–3574.
Kohno Y, Sei Y, Koshiba M, Kim HO and Jacobson KA (1996b) Induction of apoptosis in HL-60 human promyelocytic leukemia cells by adenosine A_3 receptor agonists. *Biochem Biophys Res Commun* **219**:904–910.
Koizumi S, Ikeda M, Inoue K, Nakazawa K and Inoue K (1995a) Potentiation by zinc of ATP-evoked dopamine release from rat phaeochromocytoma PC12 cells. *Brain Res* **673**:75–82.
Koizumi S, Nakazawa K and Inoue K (1995b) Inhibition by Zn^{2+} of uridine 5'-triphosphate-induced Ca^{2+}-influx but not Ca^{2+}-mobilization in rat phaeochromocytoma cells. *Br J Pharmacol* **115**:1502–1508.
Koizumi S, Watano T, Nakazawa K and Inoue K (1994) Potentiation by adenosine of ATP-evoked dopamine release via a pertussis-sensitive mechanism in rat phaeochromocytoma PC12 cells. *Br J Pharmacol* **112**:992–997.
Krishtal OA, Marchenko SM and Obukhov AG (1988a) Cationic channels activated by extracellular ATP in rat sensory neurons. *Neuroscience* **3**:995–1000.
Krishtal OA, Marchenko SM, Obukhov AG and Voltova TM (1988b) Receptors for ATP in rat sensory neurons: The structure function relationship for ligands. *Br J Pharmacol* **95**:1057–1062.
Kumagai H, Sacktor B and Filburn CR (1991) Purinergic regulation of cytosolic calcium and phosphoinositide metabolism in rat osteoblast-like osteosarcoma cells. *J Bone Miner Res* **6**:697–708.
Kurokawa M, Kirk IP, Kirkpatrick KA, Kase H and Richardson PJ (1994) Inhibition by KF17837 of adenosine A_{2A} receptor-mediated modulation of striatal GABA and ACh release. *Br J Pharmacol* **113**:43–48.
Kuroki M, Takeshige K and Minakami S (1989) ATP-induced calcium mobilization in human neutrophils. *Biochim Biophys Acta* **1012**:103–106.
Lagaud GJL, Stoclet JC and Andriantsitohaina R (1996) Calcium handling and purinoceptor subtypes involved in ATP-induced contraction in rat small mesenteric arteries. *J Physiol (Lond)* **492**:689–703.
Lai H-L, Yang T-H, Messing RO, Ching Y-H, Lin S-C and Chern Y (1997) Protein kinase C inhibits adenylyl cyclase type VI activity during desensitization of the A2a-adenosine receptor-mediated cAMP response. *J Biol Chem (Tokyo)* **272**:4970–4977.
Lambrecht G (1996) Design and pharmacology of selective P_2-purinoceptor antagonists. *J Auton Pharmacol* **16**:341–344.
Lambrecht G, Ardanuy U, Bäumert HG, Bo X, Hoyle CHV, Nickel P, Pfaff O, Ralevic V, Windscheif U, Ziganshin AU, Ziyal R, Mutschler E and Burnstock G (1996) Design and pharmacological characterization of selective P_2-purinoceptor antagonists, in *Perspectives in Receptor Research* (Giardinà D, Piergentili A and Pigini M eds) vol 24, pp 337–350, Elsevier Science, Amsterdam.
LaNoue KF and Martin LF (1994) Abnormal A_1 adenosine receptor function in genetic obesity. *FASEB J* **8**:72–80.
Lasley RD, Anderson GM and Mentzer RM Jr (1993) Ischaemic and hypoxic preconditioning enhance postischaemic recovery of function in the rat heart. *Cardiovasc Res* **27**:565–570.
Lasley RD and Mentzer RM Jr (1995) Protective effects of adenosine in the reversibly injured heart. *Ann Thorac Surg* **60**:843–846.
Lazarowski ER and Harden TK (1994) Identification of a uridine nucleotide-selective G-protein-linked receptor that activates phospholipase C. *J Biol Chem* **269**:11830–11836.
Lazarowski ER, Homolya L, Boucher RC and Harden TK (1997a) Direct demonstration of mechanically induced release of cellular UTP and its implication for uridine nucleotide receptor activation. *J Biol Chem* **272**:24348–24354.
Lazarowski ER, Paradiso AM, Watt WC, Harden TK and Boucher RC (1997b) UDP activates a mucosal-restricted receptor on human nasal epithelial cells that is distinct from the $P2Y_2$ receptor. *Proc Natl Acad Sci USA* **94**:2599–2603.
Lazarowski ER, Watt WC, Stutts MJ, Boucher RC and Harden TK (1995) Pharmacological selectivity of the cloned human P_{2U}-purinoceptor: Potent activation by diadenosine tetraphosphate. *Br J Pharmacol* **116**:1619–1627.
Lazarowski ER, Watt WC, Stutts MJ, Brown HA, Boucher RC and Harden TK (1996) Enzymatic synthesis of UTPγS, a potent hydrolysis resistant agonist of P_{2U}-purinoceptors. *Br J Pharmacol* **117**:203–209.

Le F, Townsend-Nicholson A, Baker E, Sutherland GR and Schofield PR (1996) Characterization and chromosomal localization of the human A2a adenosine receptor gene: ADORA2A. *Biochem Biophys Res Commun* **223**:461–467.

Ledent C, Vaugeois J-M, Schiffmann SN, Pedrazzini T, Yacoubi ME, Vanderhaeghen J-J, Costentin J, Heath JK, Vassart G and Parmentier M (1997) Appressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A_{2a} receptor. *Nature (Lond.)* **388**:674–678.

Lee HT, Thompson CI, Hernandez A, Lewy JL and Belloni FL (1993) Cardiac desensitization to adenosine analogs after prolonged R-PIA infusion in vivo. *Am J Physiol* **265**:H1916–H1927.

Lee PS, Squires PE, Buchan AM, Yuen BH and Leung PC (1996) P_2-purinoceptor evoked changes in intracellular calcium oscillations in single isolated human granulosa-lutein cells. *Endocrinology* **137**:3756–3761.

Lee YM, Sheu JR and Yen MH (1995) BN-063, a newly synthesized adenosine A_1 receptor agonist, attenuates myocardial reperfusion injury in rats. *Eur J Pharmacol* **297**:251–256.

Leff P, Wood BE and O'Connor SE (1990) Suramin is a slowly-equilibrating but competitive antagonist at P2X-receptors in the rabbit isolated ear artery. *Br J Pharmacol* **101**:645–649.

Léon C, Hechler B, Vial C, Leray C, Cazenave J-P and Gachet C (1997) The $P2Y_1$ receptor is an ADP receptor antagonized by ATP and expressed in platelets and megakaryoblastic cells. *FEBS Lett* **403**:26–30.

Léon C, Vial C, Cazenave J-P and Gachet C (1995) Cloning and sequencing of a human cDNA encoding endothelial $P2Y_1$ purinoceptor. *Gene* **171**:295–297.

Levens N, Beil M and Jarvis M (1991a) Renal actions of a new adenosine agonist, CGS 21680A selective for the A_2 receptor. *J Pharmacol Exp Ther* **257**:1005–1012.

Levens N, Beil M and Schulz R (1991b) Intrarenal actions of the new adenosine agonist CGS 21680A, selective for the A_2 receptor. *J Pharmacol Exp Ther* **257**: 1013–1019.

Lewis CD, Hourani SM, Long CJ and Collis MG (1994) Characterization of adenosine receptors in the rat isolated aorta. *Gen Pharmacol* **25**:1381–1387.

Lewis C, Neidhart S, Holy C, North RA, Buell G and Surprenant A (1995) Coexpression of $P2X_2$ and $P2X_3$ receptor subunits can account for ATP-gated currents in sensory neurons. *Nature (Lond.)* **377**:432–435.

Li C, Peoples RW, Li Z and Weight FF (1993) Zn^{2+} potentiates excitatory action of ATP on mammalian neurons. *Proc Natl Acad Sci USA* **90**:8264–8267.

Li C, Peoples RW and Weight FF (1996) Proton potentiation of ATP-gated ion channel responses to ATP and Zn^{2+} in rat nodose ganglion neurons. *J Neurophysiol* **76**:3048–3058.

Li C, Peoples RW and Weight FF (1997a) Mg^{2+} inhibition of ATP-activated current in rat nodose ganglion neurons: Evidence that Mg^{2+} decreases the agonist affinity of the receptor. *J Neurophysiol* **77**:3391–3395.

Li C, Peoples RW and Weight FF (1997b) Enhancement of ATP-activated current by protons in dorsal root ganglion neurons. *Pflüeg Arch Eur J Physiol* **433**:446–454.

Li J and Perl ER (1995) ATP modulation of synaptic transmission in the spinal substantia gelatinosa. *J Neurosci* **15**:3357–3365.

Li Q, Schachter JB, Harden TK and Nicholas RA (1997c) The 6H1 orphan receptor, claimed to be the p2y5 receptor, does not mediate nucleotide-promoted second messenger responses. *Biochem Biophys Res Commun* **236**:455–460.

Liang BT and Haltiwanger B (1995) Adenosine A_{2a} and A_{2b} receptors in cultured fetal chick heart cells: High- and low-affinity coupling to stimulation of myocyte contractility and cAMP accumulation. *Circ Res* **76**:242–251.

Libert F, Parmentier M, Lefort A, Dinsart C, Van Sande J, Maenhaut C, Simons MJ, Dumont JE and Vassart G (1989) Selective amplification and cloning of four new members of the G protein-coupled receptor family. *Science (Wash. DC)* **244**:569–572.

Libert F, Passage E, Parmentier M, Simons M-J, Vassart G and Mattei M-G (1991) Chromosomal mapping of A_1 and A_2 adenosine receptors, VIP receptor, and a new subtype of serotonin receptor [Erratum]. *Genomics* **11**:225–227.

Libert F, Schiffmann SN, Lefort A, Parmentier M, Gerard C, Dumont JE, Vanderhaeghen J-J and Vassart G (1991) The orphan receptor cDNA RDC7 encodes an A_1 adenosine receptor. *EMBO (Eur Mol Biol Organ) J* **10**:1677–1682.

Libert F, van Sande J, Lefort A, Czernilofsky A, Dumont JE, Vassart G, Ensinger HA and Mendla KD (1992) Cloning and functional characterization of a human A_1 adenosine receptor. *Biochem Biophys Res Commun* **187**:919–926.

Lin TA, Lustig KD, Sportiello MG, Weisman GA and Sun GY (1993) Signal transduction pathways coupled to a P_{2U} receptor in neuroblastoma × glioma (NG108–15) cells. *J Neurochem* **60**:1115–1125.

Lin WW and Chuang DM (1993) Endothelin- and ATP-induced inhibition of adenylyl cyclase activity in C6 glioma cells: Role of G_i and calcium. *Mol Pharmacol* **44**:158–165.

Lin WW and Chuang DM (1994) Different signal transduction pathways are coupled to the nucleotide receptor and the P2Y receptor in C6 glioma cells. *J Pharmacol Exp Ther* **269**:926–931.

Lin WW and Lee YT (1996) Pyrimidinoceptor-mediated activation of phospholipase C and phospholipase A_2 in RAW 264.7 macrophages. *Br J Pharmacol* **119**:261–268.

Lin Y and Phillis JW (1991) Characterization of the depression of rat cerebral cortical neurons by selective adenosine agonists. *Brain Res* **540**:307–310.

Linden J (1994) Cloned adenosine A_3 receptors: Pharmacological properties, species differences and receptor functions. *Trends Pharmacol Sci* **15**:298–306.

Linden J, Taylor HE, Robeva AS, Tucker AL, Stehle JH, Rivkees SA, Fink JS and Reppert SM (1993) Molecular cloning and functional expression of a sheep A_3 adenosine receptor with widespread tissue distribution. *Mol Pharmacol* **44**:524–532.

Lindström K, Ongini E and Fredholm BB (1996) The selective adenosine A_{2A} receptor antagonist SCH 58261 discriminates between two different binding sites for [^{3}H]-CGS 21680 in the rat brain. *Naunyn-Schmiedeberg's Arch Pharmacol* **354**: 539–541.

Liu SF, Crawley DE, Evans TW and Barnes PJ (1992) Endothelium-dependent nonadrenergic, non-cholinergic neural relaxation in guinea pig pulmonary artery. *J Pharmacol Exp Ther* **260**:541–548.

Liu SF, McCormack DG, Evans TW and Barnes PJ (1989) Evidence for two P_2-purinoceptor subtypes in human small pulmonary arteries. *Br J Pharmacol* **98**: 1014–1020.

Liu GS, Richards SC, Olsson RA, Mullane K, Walsh RS and Downey JM (1994) Evidence that the adenosine A_3 receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. *Cardiovasc Res* **28**:1057–1061.

Liu P, Wen M and Hayashi J (1995) Characterization of ATP receptor responsible for the activation of phospholipase A_2 and stimulation of prostaglandin E_2 production in thymic epithelial cells. *Biochem J* **308**:399–404.

Lohse MJ, Klotz K-N, Lindenborn-Fotinos J, Reddington M, Schwabe U and Olsson RA (1987) 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX): A selective, high affinity antagonist radioligand for A_1 adenosine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **336**:204–210.

Lohse MJ, Klotz K-N, Schwabe U, Cristalli G, Vittori S and Grifantini M (1988) 2-Chloro-N^6-cyclopentyladenosine: A highly selective agonist at A_1 adenosine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **337**:687–689.

Londos C, Cooper DMF and Wolff J (1980) Subclasses of external adenosine receptors. *Proc Natl Acad Sci USA* **77**:2551–2554.

Londos C, Honnor RC and Dhillon G (1985) cAMP-dependent protein kinase and lipolysis in rat adipocytes: III—Multiple modes of insulin regulation of lipolysis and regulation of insulin responses by adenylate cyclase regulators. *J Biol Chem* **260**:15139–15145.

Longabaugh JP, Didsbury J, Spiegel A and Stiles GL (1989) Modification of the rat adipocyte A_1 adenosine receptor-adenylate cyclase system during chronic exposure to an A_1 adenosine receptor agonist: Alterations in the quantity of $G_{s\alpha}$ and $G_{i\alpha}$ are not associated with changes in their mRNAs. *Mol Pharmacol* **36**:681–688.

Longhurst PA, Schwegel T, Folander K and Swanson R (1996) The human $P2X_1$ receptor: Molecular cloning, tissue distribution, and localization to chromosome 17. *Biochim Biophys Acta* **1308**:185–188.

Losinski A and Alexander SPH (1995) Adenosine receptor-mediated relaxation of guinea-pig precontracted, isolated trachea. *Br J Pharmacol* **116**:2425–2428.

Lupica CR, Berman RF and Jarvis MF (1991a) Chronic theophylline treatment increases adenosine A_1, but not A_2 receptor binding in the rat brain: An autoradiographic study. *Synapse* **9**:95–102.

Lupica CR, Cass WA, Zahniser NR and Dunwiddie TV (1990) Effects of the selective adenosine A2 receptor agonist CGS 21680 on in vitro electrophysiology, cAMP formation and dopamine release in rat hippocampus and striatum. *J Pharmacol Exp Ther* **252**:1134–1141.

Lupica CR, Jarvis MF and Berman RF (1991b) Chronic theophylline treatment in vivo increases high affinity adenosine A_1 receptor binding and sensitivity to exogenous adenosine in the in vitro hippocampal slice. *Brain Res* **542**:55–62.

Lustig KD, Shiau AK, Brake AJ and Julius D (1993) Expression cloning of an ATP receptor from mouse neuroblastoma cells. *Proc Natl Acad Sci USA* **90**:5113–5117.

Lustig KD, Sportiello MG, Erb L and Weisman GA (1992) A nucleotide receptor in vascular endothelial cells is specifically activated by the fully ionized forms of ATP and UTP. *Biochem J* **284**:733–739.

Luthin DR and Linden J (1995) Comparison of A_4 and A_{2a} binding sites in striatum and COS cells transfected with adenosine A_{2a} receptors. *J Pharmacol Exp Ther* **272**:511–518.

Luthje J and Ogilvie A (1983) The presence of diadenosine 5′,5‴-P^1, P^3-trisphosphate (Ap_3A) in human platelets. *Biochem Biophys Res Commun* **115**:253–260.

MacCollin M, Peterfreund RA, MacDonald M, Fink JS and Gusella J (1994) Mapping of a human A2a adenosine receptor (ADORA2) to chromosome 22. *Genomics* **20**:332–333.

Mackenzie AB, Mahaut-Smith MP and Sage SO (1996) Activation of receptor-operated cation channels via P_{2X1} not P_{2T} purinoceptors in human platelets. *J Biol Chem* **271**:2879–2881.

Maenhaut C, Van Sande J, Libert F, Abramowicz M, Parmentier M, Vanderhaegen JJ, Dumont JE, Vassart G and Schiffmann S (1990) RDC8 codes for an adenosine A_2 receptor with physiological constitutive activity. *Biochem Biophys Res Commun* **173**:1169–1178.

Maggirwar SB, Dhanraj DN, Somani SM and Ramkumar V (1994) Adenosine acts as an endogenous activator of the cellular antioxidant defense system. *Biochem Biophys Res Commun* **201**:508–515.

Mahan LC, McVittie LD, Smyk-Randall EM, Nakata H, Monsma FJ Jr, Gerfen CR and Sibley DR (1991) Cloning and expression of an A_1 adenosine receptor from rat brain. *Mol Pharmacol* **40**:1–7.

Majid MA, Okajima F and Kondo Y (1992) Characterization of ATP receptor which mediates norepinephrine release in PC12 cells. *Biochim Biophys Acta* **1136**:283–289.

Majid MA, Okajima F and Kondo Y (1993) UTP activates phospholipase C-Ca^{2+} system through a receptor different from the 53-kDa ATP receptor in PC12 cells. *Biochem Biophys Res Commun* **195**:415–421.

Makujina SR and Mustafa SJ (1993) Adenosine-5′-uronamides rapidly desensitize the adenosine A_2 receptor in coronary artery. *J Cardiovasc Pharmacol* **22**:506–509.

Makujina SR, Sabouni MH, Bhatia S, Douglas FL and Mustafa SF (1992) Vasodilatory effects of adenosine A_2 receptor agonists CGS 21680 and CGS 22492 in human vasculature. *Eur J Pharmacol* **221**:243–247.

Malam-Souley R, Seye C, Gadeau AP, Loirand G, Pillois X, Campan M, Pacaud P and Desgranges C (1996) Nucleotide receptor P_{2u} partially mediates ATP-induced cell cycle progression of aortic smooth muscle cells. *J Cell Physiol* **166**:57–65.

Malhotra J and Gupta YK (1997) Effect of adenosine receptor modulation on pentylenetetrazole-induced seizures in rats. *Br J Pharmacol* **120**:282–288.

Marin P, Tence M, Delumeau J-C, Glowinski J and Premont J (1993) Adenosine and somatostatin potentiate the α_1-adrenergic activation of phospholipase C in striatal astrocytes through a mechanism involving arachidonic acid and glutamate. *Biochem Soc Trans* **21**:1114–1119.

Markwardt F, Löhn M, Böhm T and Klapperstück M (1997) Purinoceptor-operated cationic channels in human B lymphocytes. *J Physiol (Lond)* **498**:143–151.

Marquardt DL, Walker LL and Heinemann S (1994) Cloning of two adenosine receptor subtypes from mouse bone marrow-derived mast cells. *J Immunol* **152**: 4508–4515.

Marsault R, Vigne P and Frelin C (1992) High reactivity of aortic fibroblasts to vasoactive agents: Endothelins, bradykinin and nucleotides. *Biochem Biophys Res Commun* **188**:205–208.

Martin PL (1992) Relative agonist potencies of C2-substituted analogs of adenosine: Evidence for A_{2B} receptors in the guinea pig aorta. *Eur J Pharmacol* **216**:235–242.

Martin KA, Kertesy SB and Dubyak GR (1997a) Down-regulation of P_{2U}-purinergic nucleotide receptor messenger RNA expression during in vitro differentiation of human myeloid leukocytes by phorbol esters or inflammatory activators. *Mol Pharmacol* **51**:97–108.

Martin PL, Barrett RJ, Linden J and Abraham WM (1997b) Pharmacology of 2-cyclohexylmethylidenehydrazionadenosine (WRC-0470), a novel, short-acting adenosine A_{2A} receptor agonist that produces selective coronary vasodilation. *Drug Dev Res* **40**:313–324.

Martin PL and Potts AA (1994) The endothelium of the rat renal artery plays an obligatory role in A_2 adenosine receptor-mediated relaxation induced by 5′-N-ethylcarboxamidoadenosine and N^6-cyclopentyladenosine. *J Pharmacol Exp Ther* **270**:893–899.

Martin PL, Potts AA, Sykes AM and McKenna DG (1993a) (±)-N^6-endonorbornan-2-yl-9-methyladenine (N-0861) and its enantiomers: Selective antagonists of A_1-adenosine receptors in guinea pig isolated atria. *J Pharmacol Exp Ther* **265**:201–266.

Martin PL, Ueeda M and Olsson RA (1993b) 2-Phenylethoxy-9-methyladenosine: An adenosine receptor antagonist that discriminates between A_2 adenosine receptor in the aorta and the coronary vessels from the guinea pig. *J Pharmacol Exp Ther* **265**:248–253.

Martin PL, Wysocki RJ Jr, Barrett RJ, May JM and Linden J (1996) Characterization of 8-(N-methylisopropyl)amino-N^6-(5′endohydroxy-endonorbornyl)-9-methyladenine (WRC 0571), a highly potent and selective, non-xanthine antagonist of A_1 adenosine receptors. *J Pharmacol Exp Ther* **276**:490–499.

Martin SC and Shuttleworth TJ (1995) Activation of a P_{2U} 'nucleotide' receptor in an exocrine cell. *Br J Pharmacol* **115**:321–329.

Martin TW and Michaelis K (1989) P_2-purinergic agonists stimulate phosphodiesterase cleavage of phosphatidylcholine in endothelial cells: Evidence for activation of phospholipase D. *J Biol Chem* **264**:8847–8856.

Martin W, Cusack NJ, Carleton JS and Gordon JL (1985) Specificity of the P_2-purinoceptor that mediates endothelium-dependent relaxation of the pig aorta. *Eur J Pharmacol* **108**:295–299.

Martinson EA, Johnson RA and Wells JN (1987) Potent adenosine receptor antagonists that are selective for the A_1 receptor subtype. *Mol Pharmacol* **31**:247–252.

Mason SJ, Paradiso AM and Boucher RC (1991) Regulation of transepithelial ion transport and intracellular calcium by extracellular ATP in human normal and cystic fibrosis airway epithelium. *Br J Pharmacol* **103**:1649–1656.

Mateo J, Castro E, Zwiller J, Aunis D and Miras-Portugal MT (1995) 5′-(N-ethylcarboxamido)adenosine inhibits Ca^{2+} influx and activates a protein phosphatase in bovine adrenal chromaffin cells. *J Neurochem* **64**:77–84.

Mateo J, Rotllàn P and Miras-Portugal MT (1996) Suramin: A powerful inhibitor of neural ecto-diadenosine polyphopshate hydrolase. *Br J Pharmacol* **119**:1–2.

Matherne GP, Linden J, Byford AM, Gauthier NS and Headrick JP (1997) Transgenic A_1 adenosine receptor overexpression increases myocardial resistance to ischemia. *Proc Natl Acad Sci USA* **94**:6541–6546.

Mathie RT, Alexander B, Ralevic V and Burnstock G (1991a) Adenosine-induced dilatation of the rabbit hepatic arterial bed is mediated by A_2-purinoceptors. *Br J Pharmacol* **103**:1103–1107.

Mathie RT, Ralevic V, Alexander B and Burnstock G (1991b) Nitric oxide is the mediator of ATP-induced dilatation of the rabbit hepatic arterial vascular bed. *Br J Pharmacol* **103**:1602–1606.

Mathieson JJI and Burnstock G (1985) Purine-mediated relaxation and constriction of isolated rabbit mesenteric artery are not endothelium-dependent. *Eur J Pharmacol* **118**:221–229.

Matsumoto T, Nakane T and Chiba S (1997) UTP induces vascular responses in the isolated and perfused canine epicardial coronary artery via UTP-preferring P2Y receptors. *Br J Pharmacol* **122**:1625–1632.

Maurice DH, Waldo GL, Morris AJ, Nicholas RA and Harden TK (1993) Identification of $G\alpha_{11}$ as the phospholipase C-activating G-protein of turkey erythrocytes. *Biochem J* **290**:765–770.

May JM, Martin PL and Miller JR (1991) N-0861: A selective A_1-adenosine receptor antagonist. *FASEB J* **5**:1572.

Mayfield RD, Suzuki F and Zahniser NR (1993) Adenosine A_{2a} receptor modulation of electrically evoked endogenous GABA release from slices of rat globus pallidus. *J Neurochem* **60**:2334–2337.

McCoy DE, Schwiebert EM, Karlson KH, Spielman WS and Stantin BA (1995) Identification and function of A_1 adenosine receptors in normal and cystic fibrosis human airway epithelial cells. *Am J Physiol* **268**:C1520–C1527.

McIntosh HH and Blazynski C (1994) Characterization and localization of adenosine A_2 receptors in bovine rod outer segments. *J Neurochem* **62**:992–997.

McLaren GL, Lambrecht G, Mutschler E, Bäumert HG, Sneddon P and Kennedy C (1994) Investigation of the actions of PPADS, a novel P_{2X}-purinoceptor antagonist, in the guinea-pig isolated vas deferens. *Br J Pharmacol* **111**:913–917.

McMillian MK, Soltoff SP, Cantley LC, Rudel RA and Talamo BR (1993) Two distinct cytosolic calcium responses to extracellular ATP in rat parotid acinar cells. *Br J Pharmacol* **108**:453–461.

Megson AC, Dickenson JM, Townsend-Nicholson A and Hill SJ (1995) Synergy between the inositol phosphate responses to transfected human adenosine A_1-receptors and constitutive P_2-purinoceptors in CHO-K1 cells. *Br J Pharmacol* **115**:1415–1424.

Meng F, Xie GX, Chalmers D, Morgan C, Watson SJ Jr and Akil H (1994a) Cloning and characterization of a pharmacologically distinct A_1 adenosine receptor from guinea pig brain. *Mol Brain Res* **26**:143–155.

Meng F, Xie GX, Chalmers D, Morgan C, Watson SJ Jr and Akil H (1994b) Cloning and expression of the A_{2a} adenosine receptor from guinea pig brain. *Neurochem Res* **19**:613–621.

Merkel LA, Lappe RW, Rivera LM, Cox BF and Perrone MH (1992) Demonstration of vasorelaxant activity with an A_1-selective adenosine agonist in porcine coronary artery: Involvement of potassium channels. *J Pharmacol Exp Ther* **260**:437–443.

Meyerhof W, Müller-Brechlin R and Richter D (1991) Molecular cloning of a novel putative G-protein coupled receptor expressed during rat spermiogenesis. *FEBS Lett* **284**:155–160.

Michel AD, Chau N-M, Fan T-PD, Frost EE and Humphrey PPA (1995) Evidence that [^{3}H]-α, β-methylene ATP may label an endothelial-derived cell line 5′-nucleotidase with high affinity. *Br J Pharmacol* **115**:767–774.

Michel AD and Humphrey PP (1993) Distribution and characterisation of [^{3}H]α, β-methylene ATP binding sites in the rat. *Naunyn Schmiedeberg's Arch Pharmacol* **348**:608–617.

Michel AD, Lundström KL, Buell GN, Surprenant A, Valera S and Humphrey PPA (1996) A comparison of the binding characteristics of recombinant P_{2X1} and P_{2X2} purinoceptors. *Br J Pharmacol* **118**:1806–1812.

Michel AD, Miller KJ, Ländström K, Buell GN and Humphrey PPA (1997) Radiolabeling of the rat P_{2X4} purinoceptor: Evidence for allosteric interactions of purinoceptor antagonists and monovalent cations with P_{2X} purinoceptors. *Mol Pharmacol* **51**:524–532.

Mills I and Gewirtz H (1990) Cultured vascular smooth muscle cells from porcine coronary artery possess A_1 and A_2 adenosine receptor activity. *Biochem Biophys Res Commun* **168**:1297–1302.

Minelli A, Miscetti P, Allegrucci C and Mezzasoma I (1995) Evidence of A_1 adenosine receptor on epididymal bovine spermatozoa. *Arch Biochem Biophys* **322**:272–276.

Mironov SL (1994) Metabotropic ATP receptor in hippocampal and thalamic neurons: Pharmacology and modulation of Ca^{2+} mobilizing mechanisms. *Neuropharmacology* **33**:1–13.

Miyagi Y, Kobayashi S, Nishimura J, Fukui M and Kanaide H (1996a) Dual regulation of cerebrovascular tone by UTP: P_{2U} receptor-mediated contraction and endothelium-dependent relaxation. *Br J Pharmacol* **118**:847–856.

Miyagi Y, Kobayashi S, Nishimura J, Fukui M and Kanaide H (1996b) P_{2U} receptor is linked to cytosolic Ca^{2+} transient and release of vasorelaxing factor in bovine endothelial cells in situ. *J Physiol (Lond)* **492**:751–761.

Miyahara H and Suzuki H (1987) Pre- and postjunctional effects of adenosine triphosphate on noradrenergic transmission in the rabbit ear artery. *J Physiol (Lond)* **389**:423–440.

Mizumoto H, Karasawa A and Kubo K (1993) Diuretic and renal protective effects of 8-(normadamantan-3-yl)-1,3-dipropylxanthine (KW-3902), a novel adenosine A_1-receptor antagonist, via pertussis toxin insensitive mechanism. *J Pharmacol Exp Ther* **266**:200–206.

Mizumura T, Auchampach JA, Linden J, Bruns RF and Gross GJ (1996) PD 81,723, an allosteric enhancer of the A_1 adenosine receptor, lowers the threshold for ischemic preconditioning in dogs. *Circ Res* **79**:415–423.

Mockett BG, Bo X, Housley GD, Thorne PR and Burnstock G (1995) Autoradiographic labelling of P_2 purinoceptors in the guinea-pig cochlea. *Hear Res* **84**:177–193.

Mogul DJ, Adams ME and Fox AP (1993) Differential activation of adenosine receptors decreases N-type but potentiates P-type Ca^{2+} current in hippocampal CA3 neurons. *Neuron* **10**:327–334.

Monitto CL, Levitt RC, Disilvestre D and Holroyd KJ (1995) Localization of the A_3 adenosine receptor gene (ADORA3) to human chromosome 1p. *Genomics* **26**:637–638.

Monopoli A, Conti A, Zocchi C, Casati C, Volpini R, Cristalli G and Ongini E (1994) Pharmacology of the new selective A_{2A} adenosine receptor agonist 2-hexynyl-5′-N-ethylcarboxamidoadenosine. *Arzneim-Forsch* **44**:1296–1304.

Montserrat C, Merten M and Figarella C (1996) Defective ATP-dependent mucin secretion by cystic fibrosis pancreatic epithelial cells. *FEBS Lett* **393**:264–268.

Morimoto H, Yamashita M, Imazumi K, Ochi T, Seki N, Mizuhara H, Fujii T and Senoh H (1993) Effects of adenosine A_2 receptor agonists on the excitation of capsaicin-sensitive afferent sensory nerves in airway tissues. *Eur J Pharmacol* **240**:121–126.

Motin L and Bennett MR (1995) Effect of P_2-purinoceptor antagonists on glutamatergic transmission in the rat hippocampus. *Br J Pharmacol* **115**:1276–1280.

Motte S, Pirotton S and Boeynaems JM (1993a) Heterogeneity of ATP receptors in aortic endothelial cells: Involvement of P_{2y} and P_{2u} receptors in inositol phosphate response. *Circ Res* **172**:504–510.

Motte S, Pirotton S and Boeynaems JM (1993b) Evidence that a form of ATP uncomplexed with divalent cations is the ligand of P_{2y} and nucleotide/P_{2u} receptors on aortic endothelial cells. *Br J Pharmacol* **109**:967–971.

Mozrymas JW and Ruzzier F (1992) ATP activates junctional and extrajunctional acetylcholine receptor channels in isolated adult rat muscle fibres. *Neurosci Lett* **139**:217–220.

Müller CE, Geis U, Grahner B, Lanzner W and Eger K (1996a) Chiral pyrrolo[2,3-d]pyrimidine and pyrimido[4,5-b]indole derivatives: Structure-activity relationships of potent, highly stereoselective A_1-adenosine receptor antagonists. *J Med Chem* **39**:2482–2491.

Müller CE, Hipp J, Knoblauch B, Schobert U, Sauer R and Geis U (1996b) DMPX (3,7-dimethyl-1-propargylxanthine) derivatives: Structure-activity relationships of potent selective A_{2a}-adenosine receptor antagonists. *Drug Dev Res* **37**:112.

Mundell SJ, Benovic JL and Kelly E (1997) A dominant negative mutant of the G protein-coupled receptor kinase 2 selectively attenuates adenosine A2 receptor desensitization. *Mol Pharmacol* **51**:991–998.

Munger KA and Jackson EK (1994) Effects of selective A_1 receptor blockade on glomerular hemodynamics: Involvement of renin-angiotensin system. *Am J Physiol* **267**:F783–F790.

Munshi R, Debernardi MA and Brooker G (1993) P_{2U}-purinergic receptors on C6–2B

rat glioma cells: Modulation of cytosolic Ca^{2+} and cAMP levels by protein kinase C. *Mol Pharmacol* **44**:1185–1191.

Munshi R, Pang I-H, Sternweis PC and Linden J (1991) A_1 adenosine receptors of bovine brain couple to guanine nucleotide-binding proteins G_{i1}, G_{i2} and G_o. *J Biol Chem* **266**:22285–22289.

Murgia M, Hanau S, Pizzo P, Rippa M and Di Virgilio F (1993) Oxidized ATP: An irreversible inhibitor of the macrophage purinergic P_{2Z} receptor. *J Biol Chem* **268**:8199–8203.

Murgia M, Pizzo P, Steinberg TH and Di Virgilio F (1992) Characterization of the cytotoxic effect of extracellular ATP in J774 mouse macrophages. *Biochem J* **288**:897–901.

Murray TF (1982) Up-regulation of rat cortical adenosine receptors following chronic administration of theophylline. *Eur J Pharmacol* **82**:113–114.

Murrin RJA and Boarder MR (1992) Neuronal 'nucleotide' receptor linked to phospholipase C and phospholipase D? Stimulation of PC12 cells by ATP analogs and UTP. *Mol Pharmacol* **41**:561–568.

Mynlieff M and Beam KG (1994) Adenosine acting at an A_1 receptor decreases N-type calcium current in mouse motoneurons. *J Neurosci* **14**:3628–3634.

Nabekura J, Ueno S, Ogawa T and Akaike N (1995) Colocalization of ATP and nicotinic ACh receptors in the identified vagal preganglionic neuron of rat. *J Physiol (Lond)* **489**: 519–527.

Najbar AT, Li CG and Rand MJ (1996) Evidence for two distinct P_2-purinoceptors subserving contraction of the rat anococcygeus smooth muscle. *Br J Pharmacol* **118**:537–542.

Nakamura F and Strittmatter SM (1996) $P2Y_1$ purinergic receptors in sensory neurons: Contribution to touch-induced impulse generation. *Neurobiology* **93**: 10465–10470.

Nakaoka Y and Yamashita M (1995) Ca^{2+} responses to acetylcholine and adenosine triphosphate in the otocyst of chick embryo. *J Neurobiol* **28**:23–34.

Nakashima J, Ohigashi T, Brookins JW, Beckman BS, Agrawal KC and Fisher JW (1993) Effects of 5′-N-ethylcarboxamideadenosine (NECA) on erythropoietin production. *Kidney Int* **44**:734–740.

Nakazawa K (1994) ATP-activated current and its interaction with acetylcholine-activated current in rat sympathetic neurons. *J Neurosci* **14**:740–750.

Nakazawa K, Fujimori K, Takanaka A and Inoue K (1990) An ATP-activated conductance in pheochromocytoma cells and its suppression by extracellular calcium. *J Physiol (Lond)* **428**:257–272.

Nakazawa K and Hess P (1993) Block by calcium of ATP-activated channels in pheochromocytoma cells. *J Gen Physiol* **101**:377–392.

Nakazawa K and Inoue K (1992) Roles of Ca^{2+} influx through ATP-activated channels in catecholamine release from pheochromocytoma cells. *J Neurophysiol* **68**: 2026–2032.

Nakazawa K and Inoue K (1993) ATP- and acetycholine-activated channels coexisting in cell-free membrane patches from rat sympathetic neuron. *Neurosci Lett* **163**:97–100.

Nakazawa K, Inoue K, Ito K, Koizumi S and Inoue K (1995) Inhibition by suramin and reactive blue 2 of GABA and glutamate receptor channels in rat hippocampal neurons. *Naunyn-Schmiedeberg's Arch Pharmacol* **351**:202–208.

Nakazawa K, Liu M, Inoue K and Ohno Y (1997) Potent inhibition by trivalent cations of ATP-gated channels. *Eur J Pharmacol* **325**:237–243.

Nakhostine N and Lamontagne D (1993) Adenosine contributes to hypoxia-induced vasodilation through ATP-sensitive K^+ channel activation. *Am J Physiol* **265**: H1289–H1293.

Nanoff C, Waldhoer M, Roka F and Freissmuth M (1997) G protein coupling of the rat A_1-adenosine receptor-partial purification of a protein which stabilizes the receptor-G protein complex. *Neuropharmacology* **36**:1211–1219.

Neal M and Cunningham J (1994) Modulation by endogenous ATP of the light-evoked release of ACh from retinal cholinergic neurons. *Br J Pharmacol* **113**:1085–1987.

Neary JT (1996) Trophic actions of extracellular ATP on astrocytes, synergistic interactions with fibroblast growth factors and underlying signal transduction mechanisms, in *P2 Purinoceptors: Localization, Function and Transduction Mechanisms* (Chadwick DJ and Goode JA eds) pp 130–139, John Wiley & Sons, Chichester.

Neely CF and Keith IM (1995) A_1 adenosine receptor antagonists block ischemia-reperfusion injury of the lung. *Am J Physiol* **268**:L1036–L1046.

Nguyen T, Erb L, Weisman GA, Marchese A, Heng HHQ, Garrad RC, George SR, Turner JT and O'Dowd BF (1996) Cloning, expression and chromosomal localization of the uridine nucleotide receptor gene. *J Biol Chem (Tokyo)* **270**:30845–30848.

Nicholas RA, Watt WC, Lazarowski ER, Li Q and Harden K (1996) Uridine nucleotide selectivity of three phospholipase C-activating P_2 receptors: Identification of a UDP-selective, a UTP-selective, and an ATP- and UTP-specific receptor. *Mol Pharmacol* **50**:224–229.

Nicholls J, Brownhill VR and Hourani SMO (1996) Characterization of P_1-purinoceptors on rat isolated duodenum longitudinal muscle and muscularis mucosae. *Br J Pharmacol* **117**:170–174.

Nicholls J, Hourani SMO and Kitchen I (1992) Characterization of P_1-purinoceptors on rat duodenum and urinary bladder. *Br J Pharmacol* **105**:639–642.

Nicke A, Baumert HG, Rettinger J, Eichele A, Lambrecht G, Mutschler E and Schmalzing G (1998) $P2X_1$ and $P2X_3$ receptors form stable trimers: a novel structural motif of ligand-gated ion channels. *EMBO Journal* **17**:3016–3028.

Nie Z, Mei Y and Ramkumar V (1997) Short term desensitization of the A_1 adenosine receptors in DDT_1MF-2 cells. *Mol Pharmacol* **52**:456–464.

Nieber K, Poelchen W and Illes P (1997) Role of ATP in fast excitatory synaptic potentials in locus coeruleus neurons of the rat. *Br J Pharmacol* **122**:423–430.

Niiya K, Jacobson KA, Silvia SC and Olsson RA (1993) Covalent binding of a selective agonist irreversibly activates guinea pig coronary artery A_2 adenosine receptors. *Naunyn Schmiedeberg's Arch Pharmacol* **347**:521–526.

Nikodijevic O, Sarges R, Daly JW and Jacobson KA (1991) Behavioural effects of A_1- and A_2-selective adenosine agonists and antagonists: Evidence for synergism and antagonism. *J Pharmacol Exp Ther* **259**:286–294.

Nikodijevic B, Sei Y, Shin Y and Daly JW (1994) Effects of ATP and UTP in pheochromocytoma PC12 cells: Evidence for the presence of three P_2 receptors, only one of which subserves stimulation of norepinephrine release. *Cell Mol Neurobiol* **14**:27–47.

Nobles M, Revest PA, Couraud P-O and Abbott NJ (1995) Characteristics of nucleotide receptors that cause elevation of cytoplasmic calcium in immortalized rat brain endothelial cells (RBE4) and in primary cultures. *Br J Pharmacol* **115**:1245–1252.

Nonaka H, Ichimura M, Takeda M, Nonaka Y, Shimada J, Suzuki F, Yamaguchi K and Kase H (1994) KF17837 ((E)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine), a potent and selective adenosine A_2 receptor antagonist. *Eur J Pharmacol* **267**:335–341.

Nörenberg W, Cordes A, Blöhbaum G, Fröhlich R and Illes P (1997a) Coexistence of purino- and pyrimidinoceptors on activated rat microglial cells. *Br J Pharmacol* **121**:1087–1098.

Nörenberg W, Wirkner K and Illes P (1997b) Effect of adenosine and some of its structural analogs on the conductance of NMDA receptor channels in a subset of rat neostriatal neurons. *Br J Pharmacol* **122**:71–80.

North RA (1996) P2X purinoceptor plethora. *Semin Neurosci* **8**:187–194.

Nuttle LC, el-Moatassim C and Dubyak GR (1993) Expression of the pore-forming P_{2Z} purinoreceptor in *Xenopus* oocytes injected with $poly(A)^+$ RNA from murine macrophages. *Mol Pharmacol* **44**:93–101.

Nyce JW and Metzger WJ (1997) DNA antisense therapy for asthma in an animal model. *Nature (Lond.)* **385**:721–725.

Ocana M and Baeyens JM (1994) Role of ATP-sensitive K^+ channels in antinociception induced by R-PIA, an adenosine A_1 receptor agonist. *Naunyn-Schmiedeberg's Arch Pharmacol* **350**:57–62.

O'Connor SE, Dainty IA and Leff P (1991) Further subclassification of ATP receptors based on agonist studies. *Trends Pharmacol Sci* **12**:137–141.

Ogata T, Nakamura Y, Tsuji K, Shibata T, Kataoka K and Schubert P (1994) Adenosine enhances intracellular Ca^{2+} mobilization with metabotropic glutamate receptor activation by t-ACPD in cultured hippocampal astrocytes. *Neurosci Lett* **170**:5–8.

Ogawa K and Schacht J (1995) P_{2y} purinergic receptors coupled to phosphoinositide hydrolysis in tissues of the cochlear lateral wall. *Neuroreport* **6**:1538–1540.

Oglivie A (1992) Extracellular functions for Ap_nA, in *Ap 4A and Other Dinucleoside Polyphosphates* (McLennan AG ed) pp 229–273, CRC Press, London.

Ogilvie A, Bläsius R, Schulze-Lohoff E and Sterzel RB (1996) Adenine dinucleotides: A novel class of signalling molecule. *J Auton Pharmacol* **16**:325–328.

Olah ME (1997) Identification of A_{2a} adenosine receptor domains involved in selective coupling to G_s: Analysis of chimeric A1/A2a adenosine receptors. *J Biol Chem* **272**:337–344.

Olah ME, Gallo-Rodriguez C, Jacobson KA and Stiles GL (1994a) [^{125}I]AB-MECA, a high affinity radioligand for the rat A_3 adenosine receptor. *Mol Pharmacol* **45**: 978–982.

Olah ME, Jacobson KA and Stiles GL (1994b) Role of the second extracellular loop of adenosine receptors in agonist and antagonist binding: Analysis of chimeric A_1/A_3 adenosine receptors. *J Biol Chem* **269**:24692–24698.

Olah ME, Jacobson KA and Stiles GL (1994c) Identification of an adenosine receptor domain specifically involved in binding of 5′-substituted adenosine agonists. *J Biol Chem* **269**:18016–18020.

Olah ME, Ren H, Ostrowski J, Jacobson KA and Stiles GL (1992) Cloning, expression, and characterization of the unique bovine A_1 adenosine receptor. *J Biol Chem* **267**:10764–10770.

Olah ME, Ren H and Stiles GL (1995) Adenosine receptors: Protein and gene structure. *Arch Int Pharmacodyn Ther* **329**:135–150.

Olivera A, Lamas S, Rodriguez-Puyol D and Lopez-Novoa JM (1989) Adenosine induces mesangial cell contraction by an A_1-type receptor. *Kidney Int* **35**:1300–1305.

Olivera A and Lopez-Novoa JM (1992) Effect of adenosine and adenosine analogs on cyclic AMP accumulation in cultured mesangial cells and isolated glomeruli of the rat. *Br J Pharmacol* **107**:341–346.

Olivera A, Lopez-Rivas A and Lopez-Novoa JM (1992) Adenosine stimulates Ca^{2+} fluxes and increases cytosolic free Ca^{2+} in cultured rat mesangial cells. *Biochem J* **282**:871–876.

Olsson RA and Pearson JD (1990) Cardiovascular purinoceptors. *Physiol Rev* **70**: 761–845.

Ongini E and Fredholm BB (1996) Pharmacology of adenosine A_{2A} receptors. *Trends Pharmacol Sci* **17**:364–372.

O'Regan MH, Simpson RE, Perkins LM and Phillis JW (1992a) Adenosine receptor agonists inhibit the release of gamma-aminobutyric acid (GABA) from the ischemic rat cerebral cortex. *Brain Res* **582**:22–26.

O'Regan MH, Simpson RE, Perkins LM and Phillis JW (1992b) The selective A_2 adenosine receptor agonist CGS 21680 enhances excitatory transmitter amino acid release from the ischemic rat cerebral cortex. *Neurosci Lett* **138**:169–172.

Osipchuk Y and Cahalan M (1992) Cell-to-cell spread of calcium signals mediated by ATP receptors in mast cells. *Nature (Lond.)* **359**:241–244.

Pacaud P, Feolde E, Frelin C and Loirand G (1996) Characterization of the P_{2Y}-purinoceptor involved in the ATP-induced rise in cytosolic Ca^{2+} concentration in rat ileal myocytes. *Br J Pharmacol* **118**:2213–2219.

Pacaud P, Malam-Souley R, Loirand G and Desgranges C (1995) ATP raises $[Ca^{2+}]_i$ via different P_2-receptor subtypes in freshly isolated and cultured aortic myocytes. *Am J Physiol* **269**:H30–H36.

Palea S, Corsi M, Pietra C, Artibani W, Calpista A, Gaviraghi G and Trist DG (1994) ADPβS induces contraction of the human isolated urinary bladder through a purinoceptor subtype different from P_{2X} and P_{2Y}. *J Pharmacol Exp Ther* **269**:193–197.

Palea S, Pietra C, Trist DG, Artibani W, Calpista A and Corsi M (1995) Evidence for

the presence of both pre- and postjunctional P_2-purinoceptor subtypes in human isolated urinary bladder. *Br J Pharmacol* **114**:35–40.
Palmer TM, Benovic JL and Stiles GL (1995a) Agonist-dependent phosphorylation and desensitization of the rat A_3 adenosine receptor: Evidence for a G-protein-coupled receptor kinase-mediated mechanism. *J Biol Chem* **270**:29607–29613.
Palmer TM, Benovic JL and Stiles GL (1996) Molecular basis for subtype-specific desensitization of inhibitory adenosine receptors. *J Biol Chem* **271**:15272–15278.
Palmer TM, Gettys TW, Jacobson KA and Stiles GL (1994) Desensitization of the canine A_{2a} adenosine receptor: Delineation of multiple processes. *Mol Pharmacol* **45**:1082–1094.
Palmer TM, Gettys TW and Stiles GL (1995b) Differential interaction with and regulation of multiple G-proteins by the rat A_3 adenosine receptor. *J Biol Chem* **270**:16895–16902.
Palmer TM, Harris CA, Coote J and Stiles GL (1997) Induction of multiple effects on adenylyl cyclase regulation by chronic activation of the human A_3 adenosine receptor. *Mol Pharmacol* **52**:632–640.
Palmer TM and Stiles GL (1995) Adenosine receptors. *Neuropharmacology* **34**:683–694.
Palmer TM and Stiles GL (1997a) Identification of an A_{2a} adenosine receptor domain specifically responsible for mediating short-term desensitization. *Biochemistry* **36**:832–838.
Palmer TM and Stiles GL (1997b) Structure-function analysis of inhibitory adenosine receptor regulation. *Neuropharmacology* **36**:1141–1147.
Pan WJ, Osmanovic SS and Shefner SA (1995) Characterization of the adenosine A_1 receptor-activated potassium current in rat locus ceruleus neurons. *J Pharmacol Exp Ther* **273**:537–544.
Park T-J, Song S-K and Kim K-T (1997) A_{2A} adenosine receptors inhibit ATP-induced Ca^{2+} influx in PC12 cells by involving protein kinase A. *J Neurochem* **68**:2177–2185.
Parker JC and Snow RL (1972) Influence of external ATP on permeability and metabolism of dog red blood cells. *Am J Physiol* **56**:888–893.
Parr CE, Sullivan DM, Paradiso AM, Lazarowski ER, Burch LH, Olsen JC, Erb L, Weisman GA, Boucher RC and Turner JT (1995) Cloning and expression of a human P_{2U} nucleotide receptor, a target for cystic fibrosis pharmacology. *Proc Natl Acad Sci USA* **91**:3275–3279.
Parsons WJ and Stiles GL (1987) Heterologous desensitization of the inhibitory A_1 adenosine receptor-adenylate cyclase system in rat adipocytes. *J Biol Chem* **262**: 841–847.
Patel V, Brown C, Goodwin A, Wilkie N and Boarder MR (1996) Phosphorylation and activation of p42 and p44 mitogen-activated protein kinase are required for the P2 purinoceptor stimulation of endothelial prostacyclin production. *Biochem J* **320**: 221–226.
Paulais M, Baudouin-Legros M and Teulon J (1995) Extracellular ATP and UTP trigger calcium entry in mouse thick cortical ascending limbs. *Am J Physiol* **268**:F496–F502.
Pauwels RA and Joos GF (1995) Characterization of adenosine receptors in the airways. *Arch Int Pharmacodyn Ther* **329**:151–160.
Peakman MC and Hill SJ (1994) Adenosine A_{2B}-receptor-mediated cyclic AMP accumulation in primary rat astrocytes. *Br J Pharmacol* **111**:191–198.
Peakman MC and Hill SJ (1995) Adenosine A_1 receptor-mediated changes in basal and histamine-stimulated levels of intracellular calcium in primary rat astrocytes. *Br J Pharmacol* **115**:801–810.
Peakman MC and Hill SJ (1996) Adenosine A_1 receptor-mediated inhibition of cyclic AMP accumulation in type-2 but not type-1 rat astrocytes. *Eur J Pharmacol* **306**:281–289.
Pearce B and Langley D (1994) Purine- and pyrimidine-stimulated phosphoinositide breakdown and intracellular calcium mobilisation in astrocytes. *Brain Res* **660**: 329–332.
Pearce B, Murphy S, Jeremy J, Morrow C and Dandona P (1989) ATP-evoked calcium mobilization and prostanoid release from astrocytes: P_2 purinergic receptors linked to phosphoinositide hydrolysis. *J Neurochem* **52**:971–977.
Pearl RG (1994) Adenosine induces pulmonary vasodilation in the perfused rabbit lung via an adenosine A_2 receptor. *Anesth Analg* **79**:46–51.
Pelleg A and Hurt CM (1996) Mechanism of action of ATP on canine pulmonary vagal C fibre nerve terminals. *J Physiol (Lond)* **490**:265–275.
Peoples RW and Li C (1998) Inhibition of NMDA-gated ion channels by the P2 purinoceptor antagonists suramin and reactive blue 2 in mouse hippocampal neurons. *Br J Pharmacol* **124**:400–408.
Peterfreund RA, MacCollin M, Gusella J and Fink JS (1996) Characterization and expression of the human A_{2a} adenosine receptor gene. *J Neurochem* **66**:362–368.
Peterson WM, Meggyesy C, Yu K and Miller SS (1997) Extracellular ATP activates calcium signalling, ion, and fluid transport in retinal pigment epithelium. *J Neurosci* **17**:2324–2337.
Petit A and Belisle S (1995) Stimulation of intracellular calcium concentration by adenosine triphosphate and uridine 5′-triphosphate in human term placental cells: Evidence for purinergic receptors. *J Clin Endocrinol Metab* **80**:1809–1815.
Petit P, Manteghetti M and Loubatières-Mariani MM (1988) Differential effects of purinergic activation on the hydrolysis of membrane polyphosphoinositides in rat pancreatic islets. *Biochem Pharmacol* **37**:1213–1217.
Pfeilschifter J and Merriweather C (1993) Extracellular ATP and UTP activation of phospholipase D is mediated by protein kinase C-ϵ in rat renal mesangial cells. *Br J Pharmacol* **110**:847–853.
Pfister JR, Belardinelli L, Lee G, Lum RT, Milner P, Stanley WC, Linden J, Baker SP and Schreiner G (1997) Synthesis and biological evaluation of the enantiomers of the potent and selective A_1 adenosine antagonist 1,3-dipropyl-8-[2-(5,6-epoxynorbonyl)]-xanthine. *J Med Chem* **40**:1773–1778.
Phillis JW (1990) The selective A_2 agonist CGS 21680, is a potent depressant of cerebral cortical neuronal activity. *Brain Res* **509**:328–330.
Phillis JW, O'Regan MH and Perkins LM (1993a) Effect of adenosine receptor agonists on spontaneous and K^+-evoked acetylcholine release from the in vivo rat cerebral cortex. *Brain Res* **605**:293–297.
Phillis JW, Perkins LM and O'Regan MH (1993b) Potassium-evoked efflux of transmitter amino acids and purines from rat cerebral cortex. *Brain Res Bull* **31**:547–552.
Pianet I, Merle M and Labouesse J (1989) ADP and, indirectly, ATP are potent inhibitors of cAMP production in intact isoproterenol-stimulated C6 glioma cells. *Biochem Biophys Res Commun* **163**:1150–1157.
Pierce KD, Furlong TJ, Selbie LA and Shine J (1992) Molecular cloning of an adenosine receptor from human brain. *Biochem Biophys Res Commun* **187**:86–93.
Piersen CE, True CD and Wells JN (1994) A carboxyl-terminally truncated mutant and nonglycosylated A_{2a} adenosine receptors retain ligand binding. *Mol Pharmacol* **45**:861–870.
Pillai S and Bikle DD (1992) Adenosine triphosphate stimulates phosphoinositide metabolism, mobilizes intracellular calcium, and inhibits terminal differentiation of human epidermal keratinocytes. *J Clin Invest* **90**:42–51.
Pintor J, Diaz-Rey MA and Miras-Portugal MT (1993) Ap4A and ADP-β-S binding to P_2 purinoceptors present on rat brain synaptic terminals. *Br J Pharmacol* **108**: 1094–1099.
Pintor J, Gomez-Villafuertes R, Gualix J and Miras-Portugal MT (1998) Antagonistic properties of diinosine polyphosphates on dinucleotide and ATP receptors. *Drug Dev Res*, **43**:57.
Pintor J, King BF, Miras-Portugal MT and Burnstock G (1996) Selectivity and activity of adenine dinucleotides at recombinant $P2X_2$ and $P2Y_1$ purinoceptors. *Br J Pharmacol* **119**:1006–1012.
Pintor J and Miras-Portugal T (1995a) A novel receptor for diadenosine polyphosphates coupled to calcium increase in rat midbrain synaptosomes. *Br J Pharmacol* **115**:895–902.
Pintor J and Miras-Portugal T (1995b) P_2 Purinergic receptors for diadenosine polyphosphates in the nervous system. *Gen Pharmacol* **26**:229–235.
Piper AS and Hollingsworth M (1996) ATP and β,γ-methylene ATP produce relaxation of guinea-pig isolated trachealis muscle via actions at P_1 purinoceptors. *Eur J Pharmacol* **307**:183–189.
Pirotton S, Robaye B, Lagneau C and Boeynaems JM (1990) Adenine nucleotides modulate phosphatidylcholine metabolism in aortic endothelial cells. *J Cell Physiol* **142**:449–457.
Pizzo P, Murgia M, Zambon A, Zanovello P, Bronte V, Pietrobon D and Di Virgilio F (1992) Role of P_{2Z} purinergic receptors in ATP-mediated killing of tumor necrosis factor (TNF)-sensitive and TNF-resistant L929 fibroblasts. *J Immunol* **149**:3372–3378.
Porter N, Radulovacki M and Green RD (1988) Desensitization of adenosine and dopamine receptors in rat brain after treatment with adenosine analogs. *J Pharmacol Exp Ther* **244**:218–225.
Post SR, Jacobson JP and Insel PA (1996) P2 purinergic receptor agonists enhance cAMP production in Madin-Darby canine kidney epithelial cells via an autocrine/paracrine mechanism. *J Biol Chem* **271**:2029–2032.
Poucher SM, Keddie JR, Singh P, Stoggall SM, Caulkett PW, Jones G and Collis MG (1995) The in vitro pharmacology of ZM 241385, a potent, non-xanthine A_{2a} selective adenosine receptor antagonist. *Br J Pharmacol* **115**:1096–1102.
Prentice DJ and Hourani SMO (1996) Activation of multiple sites by adenosine analogs in the rat isolated aorta. *Br J Pharmacol* **118**:1509–1517.
Prentice DJ, Shankley NP and Black JW (1995) Pharmacological analysis of the interaction between purinoceptor agonists and antagonists in the guinea-pig taenia caecum. *Br J Pharmacol* **115**:549–556.
Purkiss JR and Boarder MR (1992) Stimulation of phosphatidate synthesis in endothelial cells in response to P_2-receptor activation: Evidence for phospholipase C and phospholipase D involvement, phosphatidate and diacylglycerol interconversion and the role of protein kinase C. *Biochem J* **287**:31–36.
Purkiss JR, Wilkinson GF and Boarder MR (1993) Evidence for a nucleotide receptor on adrenal medullary endothelial cells linked to phospholipase C and phospholipase D. *Br J Pharmacol* **108**:1031–1037.
Purkiss JR, Wilkinson GF and Boarder MR (1994) Differential regulation of inositol 1,4,5-trisphosphate by co-existing P_{2Y}-purinoceptors and nucleotide receptors on bovine aortic endothelial cells. *Br J Pharmacol* **111**:723–728.
Qasabian RA, Schyvens C, Owe-Young R, Killen JP, MacDonald PS, Conigrave AD and Williamson DJ (1997) Characterization of the P_2 receptors in rabbit pulmonary artery. *Br J Pharmacol* **120**:553–558.
Qian YX and McCloskey MA (1993) Activation of mast cell K^+ channels through multiple G protein-linked receptors. *Proc Natl Acad Sci USA* **90**:7844–7848.
Qu Y, Campbell DL and Strauss HC (1993) Modulation of L-type Ca^{2+} current by extracellular ATP in ferret isolated right ventricular myocytes. *J Physiol (Lond)* **471**:295–317.
Radford KM, Virginio C, Surprenant A, North RA and Kawashima E (1997) Baculovirus expression provides direct evidence for heteromeric assembly of $P2X_2$ and $P2X_3$ receptors. *J Neurosci* **17**:6529–6533.
Ralevic V and Burnstock G (1988) Actions mediated by P_2-purinoceptor subtypes in the isolated perfused mesenteric bed of the rat. *Br J Pharmacol* **95**:637–645.
Ralevic V and Burnstock G (1990) Postjunctional synergism of noradrenaline and adenosine 5′-triphosphate in the mesenteric arterial bed of the rat. *Eur J Pharmacol* **175**:291–299.
Ralevic V and Burnstock G (1991a) Roles of P_2-purinoceptors in the cardiovascular system. *Circulation* **84**:1–14.
Ralevic V and Burnstock G (1991b) Effects of purines and pyrimidines on the rat mesenteric arterial bed. *Circ Res* **69**:1583–1590.
Ralevic V and Burnstock G (1996a) Discrimination by PPADS between endothelial P_{2Y}- and P_{2U}-purinoceptors in the rat isolated mesenteric arterial bed. *Br J Pharmacol* **118**:428–434.
Ralevic V and Burnstock G (1996b) Relative contribution of P_{2U}- and P_{2Y}-purinoceptors to endothelium-dependent vasodilatation in the golden hamster isolated mesenteric arterial bed. *Br J Pharmacol* **117**:1797–1802.
Ralevic V, Burrell S, Kingdom J and Burnstock G (1997) Effects of purine and pyrimidine nucleotides on vascular tone of human placental cotyledons. *Br J Pharmacol* **121**:1121–1126.

Ralevic V, Hoyle CHV and Burnstock G (1995a) Pivotal role of phosphate chain length in vasoconstrictor versus vasodilator actions of adenine dinucleotides in rat mesenteric arteries. *J Physiol (Lond)* **483**:703–713.

Ralevic V, Lincoln J and Burnstock G (1991a) Release of vasoactive substance from endothelial cells, in *Endothelial Regulation of Vascular Tone* (Ryan US and Rubanyi GM) pp. 297–328, Marcel Dekker, New York.

Ralevic V, Mathie RT, Alexander B and Burnstock G (1991b) Characterization of P_{2X}- and P_{2Y}-purinoceptors in the rabbit hepatic arterial vasculature. *Br J Pharmacol* **103**:1108–1113.

Ralevic V, Milner P and Burnstock G (1995b) Augmented flow-induced endothelin release from the rat mesenteric arterial bed after long-term sympathectomy. *Endothelium* **3**:67–73.

Ralevic V, Milner P, Kirkpatrick KA and Burnstock G (1991c) Flow-induced release of adenosine 5'-triphosphate from endothelial cells of rat mesenteric arterial bed. *Experientia* **48**:31–34.

Ralevic V, Thomas T, Burnstock G and Spyer KM (1996) P2-purinoceptor-mediated changes in activity of neurons recorded extracellularly from the rostral ventrolateral medulla of the rat. *J Physiol (Lond)* **497**:74P.

Ralevic V, Thomas T and Spyer KM (1998) Effects of P2 purine receptor agonists microinjected into the rostral ventrolateral medulla on the cardiovascular and respiratory systems of the anaesthetized rat. *J Physiol (Lond)*, **509P**:127P.

Ramkumar V, Kwatra M, Benovic JL and Stiles GL (1993a) Functional consequences of A_1 adenosine-receptor phosphorylation by the beta-adrenergic receptor kinase. *Biochim Biophys Acta* **1179**:89–97.

Ramkumar V, Olah ME, Jacobson KA and Stiles GL (1991) Distinct pathways of desensitization of A_1- and A_2-adenosine receptors in DDT_1 MF-2 cells. *Mol Pharmacol* **40**:639–647.

Ramkumar V, Ravi R, Wilson MC, Gettys TW, Whitworth C and Rybak LP (1994) Identification of A_1 adenosine receptors in rat cochlea coupled to inhibition of adenylyl cyclase. *Am J Physiol* **267**:C731–C737.

Ramkumar V, Stiles GL, Beaven MA and Ali H (1993b) The A_3 adenosine receptor is the unique adenosine receptor which facilitates release of allergic mediators in mast cells. *J Biol Chem* **268**:16887–16890.

Rassendren F, Buell GN, Virginio C, Collo G, North RA and Surprenant A (1997) The permeabilizing ATP receptor, $P2X_7$. *J Biol Chem* **272**:5482–5486.

Read MA, Boura ALA and Walters WAW (1993) Vascular actions of purines in the foetal circulation of the human placenta. *Br J Pharmacol* **110**:454–460.

Reeve AJ and Dickenson AH (1995) The roles of spinal adenosine receptors in the control of acute and more persistent nociceptive responses of dorsal horn neurons in the anaesthetized rat. *Br J Pharmacol* **116**:2221–2228.

Reeves JJ, Coates J, Jarvis JE, Sheehan MJ and Strong P (1993) Characterization of the adenosine receptor mediating contraction in rat colonic muscularis mucosae. *Br J Pharmacol* **110**:1255–1259.

Reichsman F, Santos S and Westhead EW (1995) Two distinct ATP receptors activate calcium entry and internal calcium release in bovine chromaffin cells. *J Neurochem* **65**:2080–2086.

Reimer WJ and Dixon SJ (1992) Extracellular nucleotides elevate $[Ca^{2+}]$ in rat osteoblastic cells by interaction with two receptor subtypes. *Am J Physiol* **263**: C1040–C1048.

Reiser G (1995) Ca^{2+}- and nitric oxide-dependent stimulation of cyclic GMP synthesis in neuronal cell line induced by P_2-purinergic/pyrimidinergic receptor. *J Neurochem* **64**:61–68.

Reppert SM, Weaver DR, Stehle JH and Rivkees SA (1991) Molecular cloning and characterization of a rat A_1-receptor that is widely expressed in brain and spinal cord. *Mol Endocrinol* **5**:1037–1048.

Revan S, Montesinos MC, Naime D, Landau S and Cronstein BN (1996) Adenosine A_2 receptor occupancy regulates stimulated neutrophil function via activation of a serine/threonine protein phosphatase. *J Biol Chem* **271**:17114–17118.

Ribeiro JA and Sebastião AM (1986) Adenosine receptors and calcium: Basis for proposing a third (A_3) adenosine receptor. *Prog Neurobiol* **26**:179–209.

Ribeiro JA and Sebastião AM (1994) Further evidence for adenosine A_3 receptors [letter]. *Trends Pharmacol Sci* **15**:13.

Rice WR, Burton FM and Fiedeldey DT (1995) Cloning and expression of the alveolar Type II cell P2U-purinergic receptor. *Am J Respir Cell Mol Biol* **12**:27–32.

Rice WR and Singleton FM (1987) P_{2y}-purinoceptor regulated surfactant secretion from rat isolated alveolar type II cells is associated with mobilization of intracellular calcium. *Br J Pharmacol* **91**:833–838.

Richardson PJ, Kase H and Jenner PG (1997) Adenosine A_{2A} receptor antagonists as new agents for the treatment of Parkinson's disease. *Trends Pharmacol Sci* **18**: 338–344.

Rivkees SA (1994) Localization and characterization of adenosine receptor expression in rat testis. *Endocrinology* **135**:2307–2313.

Rivkees SA, Lasbury ME and Barbhaiya H (1995a) Identification of domains of the human A_1 adenosine receptor that are important for binding receptor subtype-selective ligands using chimeric A_1/A_{2a} adenosine receptors. *J Biol Chem* **270**: 20485–20490.

Rivkees SA, Price SL and Zhou FC (1995b) Immunohistochemical detection of A_1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum, and basal ganglia. *Brain Res* **677**:193–203.

Rivkees SA and Reppert SM (1992) RFL9 encodes an A_{2b}-adensine receptor. *Mol Endocrinol* **6**:1598–1604.

Robaye B, Boeynaems J-M and Communi D (1997) Slow desensitization of the human P2Y6 receptor. *Eur J Pharmacol* **329**:231–236.

Robertson SJ, Rae MG, Rowan EG and Kennedy C (1996) Characterization of a P_{2X}-purinoceptor in cultured neurons of the rat dorsal root ganglia. *Br J Pharmacol* **118**:951–956.

Robitaille R (1995) Purinergic receptors and their activation by endogenous purines at perisynaptic glial cells of the frog neuromuscular junction. *J Neurosci* **15**:7121–7131.

Rubino A and Burnstock G (1996) Evidence for a P_2-purinoceptor mediating vasoconstriction by UTP, ATP and related nucleotides in the isolated pulmonary vascular bed of the rat. *Br J Pharmacol* **118**:1415–1420.

Rubino A, Ralevic V and Burnstock G (1993) The P_1-purinoceptors that mediate the prejunctional inhibitory effect of adenosine on capsaicin-sensitive nonadrenergic noncholinergic neurotransmission in the rat mesenteric arterial bed are of the A_1 subtype. *J Pharmacol Exp Ther* **267**:1100–1104.

Rubino A, Ralevic V and Burnstock G (1995) Contribution of P_1- (A_{2b} subtype) and P_2-purinoceptors to the control of vascular tone in the rat isolated mesenteric arterial bed. *Br J Pharmacol* **115**:648–652.

Rugolo M, Mastrocola T, Whorle C, Rasola A, Gruenert DC, Romeo G and Galietta LJ (1993) ATP and A_1 adenosine receptor agonists mobilize intracellular calcium and activate K^+ and Cl^- currents in normal and cystic fibrosis airway epithelial cells. *J Biol Chem* **268**:24779–24784.

Sage SO, Reast R and Rink TJ (1990) ADP evokes biphasic Ca^{2+} influx in fura-2 loaded human platelets. Evidence for Ca^{2+} entry regulated by intracellular Ca^{2+} stores. *Biochem J* **265**:675–680.

Saiag B, Bodin P, Shacoori V, Catheline M, Rault B and Burnstock G (1995) Uptake and flow-induced release of uridine nucleotides from isolated vascular endothelial cells. *Endothelium* **2**:279–285.

Saiag B, Milon D, Allain H, Rault B and Driessche VD (1990) Constriction of the smooth muscle of rat tail and femoral arteries and dog saphenous vein is induced by uridine triphosphate via 'pyrimidinoceptors', and by adenosine triphosphate via P_{2x} purinoceptors. *Blood Vessels* **27**:352–364.

Saiag B, Milon D, Shacoori V, Allain H, Rault B and Van Den Driessche J (1992) Newly evidenced pyrimidinoceptors and the P_{2x} purinoceptors are present on the vascular smooth muscle and respectively mediate the UTP- and ATP-induced contractions of the dog maxillary internal vein. *Res Commun Chem Pathol Pharmacol* **76**:89–94.

Sajjadi FG, Boyle DL, Domingo RC and Firestein G (1996) cDNA cloning and characterization of A_{3i}, an alternatively spliced rat A_3 adenosine receptor variant. *FEBS Lett* **382**:125–129.

Sajjadi FG and Firestein G (1993) cDNA cloning and sequence analysis of the human A_3 adenosine receptor. *Biochim Biophys Acta Mol Cell Res* **1179**:105–107.

Sakai K, Akima M and Matsushita H (1979) Femoral vascular responses to purine and pyrimidine derivatives: Release of 5-hydroxytryptamine by purine derivatives in isolated, cross-circulated rat hind limb. *Jpn J Pharmacol* **29**:243–251.

Sakamoto J, Miura T, Goto M and Iimura O (1995) Limitation of myocardial infarct size by adenosine A_1 receptor activation is abolished by protein kinase C inhibitors in the rabbit. *Cardiovasc Res* **29**:682–688.

Salmon JE and Cronstein BN (1990) Fc gamma receptor-mediated functions in neutrophils are modulated by adenosine receptor occupancy: A_1 receptors are stimulatory and A_2 receptors are inhibitory. *J Immunol* **145**:2235–2240.

Salter MW and Hicks JL (1994) ATP-evoked increases in intracellular calcium in neurons and glia from the dorsal spinal cord. *J Neurosci* **14**:1563–1575.

Salvatore CA, Jacobson MA, Taylor HE, Linden J and Johnson RG (1993) Molecular cloning and characterization of the human A_3 adenosine receptor. *Proc Natl Acad Sci USA* **90**:10365–10369.

Santicoli P, Del Bianco E and Maggi CA (1993) Adenosine A_1 receptors mediate the presynaptic inhibition of calcitonin gene-related peptide release by adenosine in the rat spinal cord. *Eur J Pharmacol* **231**:139–142.

Sarges R, Howard HR, Browne RG, Lebel LA, Seymour PA and Koe BK (1990) 4-Amino[1,2,4]triazolo[4,3-α]-quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants. *J Med Chem* **33**: 2240–2254.

Sasaki T and Gallacher DV (1990) Extracellular ATP activates receptor-operated cation channels in mouse lacrimal acinar cells to promote calcium influx in the absence of phosphoinositide metabolism. *FEBS Lett* **264**:130–134.

Sattin A and Rall TW (1970) The effect of adenosine and adenine nucleotides on the cyclic adenosine 3',5'-monophosphate content of guinea pig cerebral cortex slices. *Mol Pharmacol* **6**:13–23.

Sawynok J and Reid A (1996) Caffeine antinociception: Role of formalin concentration and adenosine A_1 and A_2 receptors. *Eur J Pharmacol* **298**:105–111.

Scamps F and Vassort G (1994) Pharmacological profile of the ATP-mediated increase in L-type calcium current amplitude and activation of a non-specific cation current in rat ventricular cells. *Br J Pharmacol* **113**:982–986.

Schachter JB, Li Q, Boyer JL, Nicholas RA and Harden TK (1996) Second messenger cascade specificity and pharmacological selectivity of the human P_{2Y1}-purinoceptor. *Br J Pharmacol* **118**:167–173.

Schiele JO and Schwabe U (1994) Characterization of the adenosine receptor in microvascular coronary endothelial cells. *Eur J Pharmacol* **269**:51–58.

Schiemann WP and Buxton IL (1991) Adenosine A_1-receptor coupling to phosphoinositide metabolism in pregnant guinea pig myometrium. *Am J Physiol* **261**: E665–E672.

Schiemann WP, Doggwiler KO and Buxton IL (1991b) Action of adenosine in estrogen-primed nonpregnant guinea-pig myometrium: Characterisation of the smooth muscle receptor and coupling to phosphoinositide metabolism. *J Pharmacol Exp Ther* **258**:429–437.

Schiemann WP, Westfall DP and Buxton IL (1991a) Smooth muscle adenosine A_1 receptors couple to disparate effectors by distinct G proteins in pregnant myometrium. *Am J Physiol* **261**:E141–E150.

Schiffmann SN, Libert F, Vassart G, Dumont JE and Vanderhaegen J-J (1990) A cloned G protein-coupled protein with a distribution restricted to striatal medium-sized neurons: Possible relationship with D_1 dopamine receptor. *Brain Res* **519**: 333–337.

Schiffmann SN and Vanderhaeghen JJ (1993) Adenosine A_2 receptors regulate the expression of striatopallidal and striatonigral neurons. *J Neurosci* **13**:1080–1087.

Schlüter H, Offers E, Brüggemann G, Van Der Giet M, Tepel M, Nordhoff E, Karas M, Spieker C, Witzel H and Zidek W (1994) Diadenosine phosphates and the control of blood pressure. *Nature (Lond.)* **367**:186–188.

Schöfl C, Rössig L, Pötter E, Von Zur Mühlen A and Brabant G (1995) Extracellular

ATP and UTP increase cytosolic free calcium by activating a common P_{2u}-receptor in single human thyrocytes. *Biochem Biophys Res Commun* **213**:928–934.
Scholz H, Kohl C, Neumann J, Schmitz W, Seeland C and Stein B (1993) Inotrophic actions of adenosine derivatives in the mammalian heart. *Drug Dev Res* **28**:277–282.
Scholz KP and Miller RJ (1991) Analysis of adenosine actions on Ca^{2+} currents and synaptic transmission in cultured rat hippocampal pyramidal neurons. *J Physiol (Lond)* **435**:373–393.
Schulze-Lohoff E, Bitzer M, Ogilvie A and Sterzel RB (1995) P_{2U}-purinergic receptor activation mediates inhibition of cAMP accumulation in cultured renal mesangial cells. *Renal Physiol Biochem* **18**:219–230.
Schulze-Lohoff E, Zanner S, Ogilvie A and Sterzel RB (1992) Extracellular ATP stimulates proliferation of cultured mesangial cells via P_2-purinergic receptors. *Am J Physiol* **263**:F374–F383.
Sebastião AM and Ribeiro JA (1996) Adenosine A_2 receptor-mediated excitatory actions on the nervous system. *Prog Neurobiol* **48**:167–189.
Séguéla P, Haghighi A, Soghomonian J-J and Cooper E (1996) A novel neuronal P_{2X} ATP receptor ion channel with widespread distribution in the brain. *J Neurosci* **16**:448–455.
Seifert R, Burde R and Schultz G (1989a) Activation of NADPH oxidase by purine and pyrimidine nucleotides involves G-proteins and is potentiated by chemotactic factors. *Biochem J* **259**:813–819.
Seifert R and Schultz G (1989) Involvement of pyrimidinoceptors in the regulation of cell functions by uridine and by uracil nucleotides. *Trends Pharmacol Sci* **10**:365–369.
Seifert R, Wenzel K, Eckstein F and Schultz G (1989b) Purine and pyrimidine nucleotides potentiate activation of NADPH oxidase and degranulation by chemotactic peptides and induce aggregation of human neutrophils via G-proteins. *Eur J Biochem* **181**:277–285.
Sexl V, Mancusi G, Höller C, Gloria-Maercker E, Schütz W and Freissmuth M (1997) Stimulation of the mitogen-activated protein kinase via the A_{2A}-adenosine receptor in primary human endothelial cells. *J Biol Chem* **272**:5792–5799.
Seye CI, Gadeau AP and Desgranges C (1996) Direct submission of U56839 to Genbank.
Shen MR, Linden J, Chen SS and Wu SN (1993) Identification of adenosine receptors in human spermatozoa. *Clin Exp Pharmacol Physiol* **20**:527–534.
Shen K-Z and North RA (1993) Excitation of rat locus coeruleus neurons by adenosine 5′-triphosphate: Ionic mechanism and receptor characterization. *J Neurosci* **13**:894–899.
Shi D, Nikodijevic O, Jacobson KA and Daly JW (1993) Chronic caffeine alters the density of adenosine, adrenergic, cholinergic, GABA, and serotonin receptors and calcium channels in mouse brain. *Cell Mol Neurobiol* **13**:247–261.
Shi D, Nikodijevic O, Jacobson KA and Daly JW (1994) Effects of chronic caffeine on adenosine, dopamine and acetylcholine systems in mice. *Arch Int Pharmacodyn Ther* **328**:261–287.
Shi XP, Yin KC and Gardell SJ (1995) Human erythroleukemic (HEL) cells express a platelet P_{2T}-like ADP receptor. *Thromb Res* **77**:235–247.
Shimada J, Suzuki F, Nonaka H, Ishii A and Ichikawa S (1992) (E)-1,3-dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthines: Potent and selective adenosine A_2 antagonists. *J Med Chem* **35**:2342–2345.
Shimada J, Suzuki F, Nonaka H, Karasawa A, Mizumoto H, Ohno T, Kubo K and Ishii A (1991) 8-(dicyclopropylmethyl)-1,3,-Dipropylxanthine: A potent and selective adenosine A_1 antagonist with renal and diuretic protective properties. *J Med Chem* **34**:466–469.
Shinozuka K, Bjur RA and Westfall DP (1988) Characterization of prejunctional purinoceptors on adrenergic nerves of the rat caudal artery. *Naunyn-Schmiedeberg's Arch Pharmacol* **338**:221–227.
Siddiqi SM, Jacobson KA, Esker JL, Olah ME, Ji XD, Melman N, Tiwari KN, Secrist JA III, Schneller SW, Cristalli G, Stileo GL, Johnson CR and Ijzerman AP. (1995) Search for new purine- and ribose-modified adenosine analogs as selective agonists and antagonists at adenosine receptors. *J Med Chem* **38**:1174–1188.
Silinsky EM and Gerzanich V (1993) On the excitatory effects of ATP and its role as a neurotransmitter in coeliac neurons of the guinea-pig. *J Physiol (Lond)* **464**:197–212.
Silinsky EM, Gerzanich V and Vatner SM (1992) ATP mediates excitatory synaptic transmission in mammalian neurons. *Br J Pharmacol* **106**:762–763.
Simon J, Kidd EJ, Smith FM, Chessell IP, Murrell-Lagnado R, Humphrey PPA and Barnard EA (1997) Localization and functional expression of splice variants of the $P2X_2$ receptor. *Mol Pharmacol* **52**:237–248.
Simon J, Webb TE and Barnard EA (1995) Characterization of a P_{2Y} purinoceptor in the brain. *Pharmacol Toxicol* **76**:302–307.
Simonsen U, García-Sacristàn A and Prieto D (1997) Involvement of ATP in the non-adrenergic non-cholinergic inhibitory neurotransmission of lamb isolated coronary small arteries. *Br J Pharmacol* **120**:411–420.
Sipma H, Den Hertog A and Nelemans A (1994) The phospholipase C activating P_{2U} purinoceptor also inhibits cyclic AMP formation in DDT1 MF-2 smooth muscle cells. *Eur J Pharmacol* **268**:431–437.
Sistare FD, Rosenzweig BA and Contrera JG (1995) P2 purinergic receptors potentiate parathyroid hormone receptor-mediated increases in intracellular calcium and inositol trisphosphate in UMR-106 rat osteoblasts. *Endocrinology* **136**:4489–4497.
Sistare FD, Rosenzweig BA, Contrera JG and Jordan B (1994) Separate P_{2T} and P_{2U} purinergic receptors with similar second messenger signaling pathways in UMR-106 osteoblasts. *J Pharmacol Exp Ther* **269**:1049–1061.
Snaprud P, Gerwins P, Caron MG, Libert F, Persson H, Fredholm BB and Fuxe K (1994) A_{2a}/D_2 receptor interactions are not observed in COS-7 cells transiently transfected with dopamine D_2 and adenosine A_{2a} receptor cDNA. *Biochem Pharmacol* **48**:2043–2047.
Sneddon P and Burnstock G (1984) ATP as a co-transmitter in rat tail artery. *Eur J Pharmacol* **106**:149–152.
Sobrevia L, Yudilevich DL and Mann GE (1997) Activation of A_2-purinoceptors by adenosine stimulates L-arginine transport (system y^+) and nitric oxide synthesis in human fetal endothelial cells. *J Physiol (Lond)* **499**:135–140.
Soltoff SP, McMillian MK and Talamo BR (1992) ATP activates a cation-permeable pathway in rat parotid acinar cells. *Am J Physiol* **262**:C934–C940.
Soltoff SP, McMillian MK, Talamo BR and Cantley LC (1993) Blockade of ATP binding site of P_2 purinoceptors in rat parotid acinar cells by isothiocyanate compounds. *Biochem Pharmacol* **45**:1936–1940.
Somasundaram B and Mahaut-Smith MP (1994) Three cation influx currents activated by purinergic receptor stimulation in rat megakaryocytes. *J Physiol (Lond)* **480**:225–231.
Soslau G, Mckenzie RJ, Brodsky I and Devlin TM (1995) Extracellular ATP inhibits agonist-induced mobilization of internal calcium in human platelets. *Biochim Biophys Acta* **1268**:73–80.
Soto F, Garcia-Guzman M, Gomez-Hernandez JM, Hollmann M, Karschin C and Stühmer P (1996a) $P2X_4$: An ATP-activated ionotropic receptor cloned from rat brain. *Proc Natl Acad Sci USA* **93**:3684–3688.
Soto F, Garcia-Guzman M, Karschin C and Stühmer W (1996b) Cloning and tissue distribution of a novel P2x receptor from rat brain. *Biochem Biophys Res Commun* **223**:456–460.
Southey MC, Hammet F, Hutchins AM, Paidhungat M, Somers GR and Venter DJ (1996) Molecular cloning and sequencing of a novel human P2 nucleotide receptor. *Biochim Biophys Acta* **1309**:77–80.
Sperlagh B and Vizi ES (1991) Effect of presynaptic stimulation on transmitter release. *J Neurochem* **56**:1466–1470.
Stam NJ, Klomp J, Van De Heuvel M and Olijve W (1996) Molecular cloning and characterization of a novel orphan receptor (P_{2P}) expressed in human pancreas that shows high structural homology to the P_{2U} purinoceptor. *Febs Lett* **384**:260–264.
Stambaugh K, Jacobson KA, Jiang J-L and Liang BT (1997) A novel cardioprotective function of adenosine A_1 and A_3 receptors during prolonged stimulated ischemia. *Am J Physiol* **273**:H501–H505.
Stanley JC, Markovic J, Gutknecht AM and Lozeman FJ (1987) Stimulation of glycogenolysis in isolated hepatocytes by adenosine and one of its analogs is inhibited by caffeine. *Biochem J* **247**:779–783.
Stehle JH, Rivkees SA, Lee JJ, Weaver DR, Deeds JD and Reppert SM (1992) Molecular cloning and expression of the cDNA for a novel A_2-adenosine receptor subtype. *Mol Endocrinol* **6**:384–393.
Steinberg TH and Di Virgilio F (1991) Cell-mediated cytotoxicity: ATP as an effector and the role of target cells. *Curr Opin Immunol* **3**:71–75.
Steinberg TH, Newman AS, Swanson JA and Silverstein SC (1987) ATP^{4-} permeabilizes the plasma membrane of mouse macrophages to fluorescent dyes. *J Biol Chem* **262**:8884–8888.
Steinhorn RH, Morin FC III, Van Wylen DGL, Gugino SF, Giese EC and Russell JA (1994) Endothelium-dependent relaxations to adenosine in juvenile rabbit pulmonary arteries and veins. *Am J Physiol* **266**:H2001–H2006.
Stoggall SM and Shaw JS (1990) The coexistence of adenosine A_1 and A_2 receptors in guinea-pig aorta. *Eur J Pharmacol* **190**:329–335.
Stone TW (1991) *Adenosine in the Nervous System*, Academic Press, London.
Stout JG and Kirley TL (1995) Inhibition of purified chicken gizzard smooth muscle ecto-ATPase by P_2-purinoceptor antagonists. *Biochem Mol Biol Int* **36**:927–934.
Strickler J, Jacobson KA and Liang BT (1996) Direct preconditioning of cultured chick ventricular myocytes: Novel functions of cardiac adenosine A_{2a} and A_3 receptors. *J Clin Invest* **98**:1773–1779.
Strøbæk D, Olesen S-P, Christopersen P and Dissing S (1996) P_2-purinoceptor-mediated formation of inositol phosphates and intracellular Ca^{2+} transients in human coronary artery smooth muscle cells. *Br J Pharmacol* **118**:1645–1652.
Strohmeier GR, Reppert SM, Lencer WI and Madara JL (1995) The A_{2b} adenosine receptor mediates cAMP responses to adenosine receptor agonists in human intestinal epithelia. *J Biol Chem* **270**:2387–2394.
Stutts MJ, Chinet TC, Mason SJ, Fullton JM, Clarke LL and Boucher RC (1992) Regulation of Cl^- channels in normal and cystic fibrosis airway epithelial cells by extracellular ATP. *Proc Natl Acad Sci USA* **89**:1621–1625.
Sugioka M, Fukuda Y and Yamashita M (1996) Ca^{2+} responses to ATP via purinoceptors in the early embryonic chick retina. *J Physiol (Lond)* **493**:855–863.
Suh B-C, Son JH, Joh TH and Kim K-T (1997) Two distinct P_2 purinergic receptors, P_{2Y} and P_{2U}, are coupled to phospholipase C in mouse pineal gland tumor cells. *J Neurochem* **68**:1622–1632.
Sun M-K, Wahlestedt C and Reis DJ (1992) Action of externally applied ATP on rat reticulospinal vasomotor neurons. *Eur J Pharmacol* **224**:93–96.
Sun X-P and Stanley EF (1996) An ATP-activated, ligand-gated ion channel on a cholinergic presynaptic nerve terminal. *Proc Natl Acad Sci USA* **93**:1859–1863.
Surprenant A (1996) Functional properties of native and cloned P2X receptors, in *P2 Purinoceptors: Localization, Function and Transduction Mechanisms* (Chadwick DJ and Goode JA eds) pp 208–222, John Wiley & Sons, Chichester.
Surprenant A, Rassendren FA, Kawashima E, North RA and Buell G (1996) The cytolytic P2z receptor for extracellular ATP identified as a P2X receptor ($P2X_7$). *Science (Wash. DC)* **272**:735–738.
Suzuki H and Kokubun S (1994) Subtypes of purinoceptors in rat and dog urinary bladder smooth muscle. *Br J Pharmacol* **112**:117–122.
Svenningsson P, Le Moine C, Kull B, Sunahara R, Bloch B and Fredholm BB (1997) Cellular expression of adenosine A_{2A} receptor messenger RNA in the rat central nervous system with special reference to dopamine innervated areas. *Neuroscience* **80**:1171–1185.
Svichar N, Shmigol A, Verkhratsky A and Kostyuk P (1997) ATP induces Ca^{2+} release from IP_3-sensitive Ca^{2+} stores exclusively in large DRG neurons. *Neuroreport* **8**:1555–1559.
Szentmiklósi AJ, Ujfalusi A, Cseppento A, Nosztray K, Kovacs P and Szabo JZ (1995) Adenosine receptors mediate both contractile and relaxant effects of adenosine in main pulmonary artery of guinea pigs. *Naunyn-Schmiedeberg's Arch Pharmacol* **351**:417–425.
Takeda M, Kawamura T, Kobayashi M and Endou H (1996) ATP-induced calcium

mobilization in glomerular mesangial cells is mediated by P_{2U} purinoceptor. *Biochem Mol Biol Int* **39**:1193–1200.
Tatham PER, Cusack NJ and Gomperts BD (1988) Characterisation of ATP^{4-} receptor that mediates permeabilisation of rat mast cells. *Eur J Pharmacol* **147**:13–21.
Tatham PER and Lindau M (1990) ATP-induced pore formation in the plasma membrane of rat peritoneal mast cells. *J Gen Physiol* **95**:459–476.
Terai T, Kita Y, Kusunoki T, Shimazaki T, Ando T, Horiai H, Akahane A, Shiokawa Y and Yoshida K (1995) A novel non-xanthine adenosine A_1 receptor antagonist. *Eur J Pharmacol* **279**:217–225.
Thompson RD, Secunda S, Daly JW and Olsson RA (1991) Activity of N^6-substituted 2-chloroadenosines at A_1 and A_2-adenosine receptors. *J Med Chem* **34**:3388–3390.
Todorov L, Bjur RA and Westfall DP (1994) Inhibitory and facilitatory effects of purines on transmitter release from sympathetic nerves. *J Pharmacol Exp Ther* **268**:985–989.
Tokuyama Y, Hara M, Jones EMC, Fan Z and Bell GI (1995) Cloning of rat and mouse P_{2Y} purinoceptors. *Biochem Biophys Res Commun* **211**:211–218.
Tokuyama Y, Mereu L, Chen X, Rouard M and Bell GI (1996a) Direct submission of U49395 to Genbank.
Tokuyama Y, Mereu L, Chen X, Rouard M and Bell GI (1996b) Direct submission of U49396 to Genbank.
Townsend-Nicholson A, Baker E, Schofield PR and Sutherland GR (1995a) Localization of the adenosine A_1 receptor subtype gene (ADORA1) to chromosome 1q32.1. *Genomics* **26**:423–425.
Townsend-Nicholson A, Baker E, Sutherland GR and Schofield PR (1995b) Localization of the adenosine A2b receptor subtype gene (ADORA2B) to chromosome 17p11.2-p12 by FISH and PCR screening of somatic cell hybrids. *Genomics* **25**: 605–607.
Townsend-Nicholson A and Schofield PR (1994) A threonine residue in the seventh transmembrane domain of the human A_1 adenosine receptor mediates specific agonist binding. *J Biol Chem* **269**:2373–2376.
Townsend-Nicholson A and Shine J (1992) Molecular cloning and characterization of a human brain A_1 adenosine receptor cDNA. *Mol Brain Res* **16**:365–370.
Tracey WR, Magee W, Masamune H, Kennedy SP, Knight DR, Buchholz RA and Hill RJ (1997) Selective adenosine A_3 receptor stimulation reduces ischemic myocardial injury in the rabbit heart. *Cardiovasc Res* **33**:410–415.
Traversa U, Rosati AM, Florio C and Vertua R (1994) Effects of chronic administration of adenosine antagonists on adenosine A_1 and A_{2a} receptors in mouse brain. *In Vivo* **8**:1073–1078.
Trezise DJ, Bell NJ, Kennedy I and Humphrey PPA (1994a) Effects of divalent cations on the potency of ATP and related agonists in the rat isolated vagus nerve: Implications for P_2 purinoceptor classification. *Br J Pharmacol* **113**:463–470.
Trezise DJ, Bell NJ, Khakh BS, Michel AD and Humphrey PA (1994b) P_2 purinoceptor antagonist properties of pyridoxal-5-phosphate. *Eur J Pharmacol* **259**:295–300.
Trezise DJ, Kennedy I and Humphrey PPA (1994c) The use of antagonists to characterize the receptors mediating depolarization of the rat isolated vagus nerve by α,β-methylene adenosine 5′-triphosphate. *Br J Pharmacol* **112**:282–288.
Trezise DJ, Michel AD, Grahames CB, Khakh BS, Surprenant A and Humphrey PP (1995) The selective P_{2X} purinoceptor agonist, β,γ-methylene-L-adenosine 5′-triphosphate, discriminates between smooth muscle and neuronal P_{2X} purinoceptors. *Naunyn Schmiedeberg's Arch Pharmacol* **351**:603–609.
Trivedi BK and Bruns RF (1988) [1,2,4]-Triazolo[4,3-α]-quinoxalin-4-amines: A new class of A_1 receptor selective adenosine antagonists. *J Med Chem* **31**:1011–1014.
Trussell LO and Jackson MB (1985) Adenosine-activated potassium conductance in cultured striatal neurons. *Proc Natl Acad Sci USA* **82**:4857–4861.
Tschöpl M, Harms L, Nörenberg W and Illes P (1992) Excitatory effects of adenosine 5′-triphosphate on rat locus coeruleus neurons. *Eur J Pharmacol* **213**:71–77.
Tsuchida A, Liu GS, Wilborn WH and Downey JM (1993) Pretreatment with the adenosine A_1 selective agonist, 2-chloro-N^6-cyclopentyladenosine (CCPA), causes a sustained limitation of infarct size in rabbits. *Cardiovasc Res* **27**:652–656.
Tsuchida A, Thompson R, Olsson RA and Downey JM (1994) The anti-infarct effect of an adenosine A_1-selective agonist is diminished after prolonged infusion as is the cardioprotective effect of ischaemic preconditioning in rabbit heart. *J Mol Cell Biol* **26**:303–311.
Tucker AL and Linden J (1993) Cloned receptors and cardiovascular responses to adenosine. *Cardiovasc Res* **27**:62–67.
Tucker AL, Linden J, Robeva AS, D'Angelo DD and Lynch KR (1992) Cloning and expression of a bovine adenosine A_1 receptor cDNA. *FEBS Lett* **297**:107–111.
Turner JT, Weisman GA and Camden JM (1997) Upregulation of $P2Y_2$ nucleotide receptors in rat salivary gland cells during short-term culture. *Am J Physiol* **273**:C1100–C1107.
Ueno S, Harata N, Inoue K and Akaike N (1992) ATP-gated current in dissociated rat nucleus solitarii neurons. *J Neurophysiol* **68**:778–785.
Umemiya M and Berger AJ (1994) Activation of adenosine A_1 and A_2 receptors differentially modulates calcium channels and glycinergic synaptic transmission in rat brainstem. *Neuron* **13**:1439–1446.
Uneyama H, Uneyama C, Ebihara S and Akaike N (1994) Suramin and reactive blue 2 are antagonists for a newly identified purinoceptor on rat megakaryocyte. *Br J Pharmacol* **111**:245–249.
Urquilla PR (1978) Prolonged contraction of isolated human and canine cerebral arteries induced by uridine 5′-triphosphate. *Stroke* **9**:133–6.
Usune S, Katsuragi T and Furukawa T (1996) Effects of PPADS and suramin on contractions and cytoplasmic Ca^{2+} changes evoked by AP_4A, ATP and α,β-methylene ATP in guinea-pig urinary bladder. *Br J Pharmacol* **117**:698–702.
Vahlensieck U, Boknik P, Knapp J, Linck B, Müller FU, Neumann J, Herzig S, Schlüter H, Zidek W, Deng MC, Scheld HH and Schmitz W (1996) Negative chronotropic and ionotropic effects exerted by diadenosine hexaphosphate (Ap_6A) via A_1-adenosine receptors. *Br J Pharmacol* **119**:835–844.
Valeins H, Merle M and Labouesse J (1992) Pre-steady state study of the β-adrenergic and purinergic receptor interaction in C6 cell membranes: Undelayed balance between positive and negative coupling to adenylate cyclase. *Mol Pharmacol* **42**:1033–1041.
Valera S, Hussy N, Evans RJ, Adami N, North RA, Surprenant A and Buell G (1994) A new class of ligand-gated ion channel defined by P2X receptor for extracellular ATP. *Nature (Lond.)* **371**:516–519.
Valera S, Talbot F, Evans RJ, Gos A, Antonarakis SE, Morris MA and Buell GN (1995) Characterization and chromosomal localization of a human P2X receptor from the urinary bladder. *Receptors Channels* **3**:283–289.
Valera S, Talbot F, Evans RJ, Gos A, Antonarakis SE, Morris MA and Buell GN (1996) Direct submission of X84896 to Genbank.
Van Beuren M, Bijlsma JA, Boer P, Van Rijn HJ and Koomans HA (1993) Natriuretic and hypotensive effect of adenosine-1 blockade in essential hypertension. *Hypertension* **22**:728–734.
Van Calker D, Muller M and Hamprecht B (1978) Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells. *Nature (Lond.)* **276**:839–841.
Van Calker D, Muller M and Hamprecht B (1979) Adenosine regulates via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *J Neurochem* **33**:999–1005.
Van der Ploeg I, Ahlberg S, Parkinson FE, Olsson RA and Fredholm BB (1996) Functional characterization of adenosine A2 receptors in Jurkat cells and PC12 cells using adenosine receptor agonists. *Naunyn Schmiedeberg's Arch Pharmacol* **353**:250–260.
Van der Ploeg I, Parkinson FE and Fredhom BB (1992) The effect of pertussis toxin on radioligand binding to rat brain adenosine A_1 receptors. *J Neurochem* **58**:1221–1229.
Van Galen PJM, Nissen P, Van Wijngaarden I, Ijzerman AP and Soudin W (1991) 1*H*-imidazo[4,5-*c*]quinolin-4-amines: Novel non-xanthine adenosine antagonists. *J Med Chem* **34**:1202–1206.
Van Galen PJ, Van Bergen AH, Gallo-Rodriguez C, Melman N, Olah ME, Ijzerman AP and Stiles GL (1994) A binding site model and structure-activity relationships for the rat A_3 adenosine receptor. *Mol Pharmacol* **45**:1101–1111.
Van Rhee AM, Fischer B, Van Galen PJM and Jacobson KA (1995) Modelling the P_{2Y} purinoceptor using rhodopsin as template. *Drug Des Discov* **13**:133–154.
Van Winkle DM, Chien GL, Wolff RA, Soifer BE, Kuzume K and Davis RF (1994) Cardioprotection provided by adenosine receptor activation is abolished by blockade of the KATP channel. *Am J Physiol* **266**:H829–H839.
Venkova K and Krier J (1993) Stimulation of lumbar sympathetic nerves evokes contractions of cat colon circular muscle mediated by ATP and noradrenaline. *Br J Pharmacol* **110**:1260–1270.
Verghese MW, Kneisler TB and Boucheron JA (1996) P_{2U} agonists induce chemotaxis and actin polymerization in human neutrophils and differentiated HL60 cells. *J Biol Chem* **271**:15597–15601.
Vials A and Burnstock G (1993) A_2-purinoceptor-mediated relaxation in the guinea-pig coronary vasculature: A role for nitric oxide. *Br J Pharmacol* **109**:424–429.
Vigne P, Pacaud P, Urbach V, Feolde E, Breittmayer JP and Frelin C (1996) The effect of PPADS as an antagonist of inositol (1, 4, 5)trisphosphate induced intracellular calcium mobilization. *Br J Pharmacol* **119**:360–364.
Virginio C, Church D, North RA and Surprenant A (1997) Effects of divalent cations, protons and calmidazolium at the rat $P2X_7$ receptor. *Neuropharmacology* **36**:1285–1294.
Vittet D, Mathieu M-N, Launay J-M and Chevillard C (1992) Platelet receptor expression on three human megakaryoblast-like cell lines. *Exp Hematol* **20**:1129–1134.
Von Kügelgen I, Bultmann R and Starke K (1990) Interaction of adenine nucleotides, UTP and suramin in mouse vas deferens: Suramin-sensitive and suramin-insensitive components in the contractile effect of ATP. *Naunyn-Schmiedeberg's Arch Pharmacol* **342**:198–205.
Von Kügelgen I, Häussinger D and Starke K (1987) Evidence for a vasoconstriction-mediating receptor for UTP, distinct from the P_2 purinoceptor, in rabbit ear artery. *Naunyn-Schmiedeberg's Arch Pharmacol* **336**:556–560.
Von Kügelgen I, Krumme B, Schaible U and Schollmeyer PJ (1995a) Vasoconstrictor responses to the P_{2X}-purinoceptor agonist β,γ-methylene-L-ATP in human cutaneous and renal blood vessels. *Br J Pharmacol* **116**:1932–1936.
Von Kügelgen I, Kurz K and Starke K (1994) P_2-purinoceptor-mediated autoinhibition of sympathetic transmitter release in mouse and rat vas deferens. *Naunyn-Schmiedeberg's Arch Pharmacol* **349**:125–134.
Von Kügelgen I, Späth L and Starke K (1992) Stable adenine nucleotides inhibit [^{3}H]-noradrenaline release in rabbit brain cortex slices by direct action at presynaptic adenosine A_1-receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **346**:187–196.
Von Kügelgen I and Starke K (1990) Evidence for two separate vasoconstriction-mediating nucleotide receptors, both distinct from the P2X-receptor, in rabbit basilar artery: A receptor for pyrimidine nucleotides and a receptor for purine nucleotides. *Naunyn-Schmiedeberg's Arch Pharmacol* **341**:538–546.
Von Kügelgen I and Starke K (1991) Noradrenaline-ATP cotransmission in the sympathetic nervous system. *Trends Pharmacol Sci* **12**:319–324.
Von Kügelgen I, Stoffel D, Schobert A and Starke K (1996) P_2-purinoceptors on postganglionic sympathetic neurons. *J Auton Pharmacol* **16**:413–416.
Von Kügelgen I, Stoffel D and Starke K (1995b) P_2-purinoceptor-mediated inhibition of noradrenaline release in rat atria. *Br J Pharmacol* **115**:247–254.
Von Lubitz DKJE, Lin RC-S, Popik P, Carter MF and Jacobson KA (1994) Adenosine A_3 receptor stimulation and cerebral ischemia. *Eur J Pharmacol* **263**:59–67.
Von Lubitz DKJE, Lin RC-S, Sei Y, Boyd M, Abbracchio M, Bischofberger N and Jacobson KA (1996) Adenosine A_3 receptors and ischemic brain injury: A hope or a disaster? *Drug Dev Res* **37**:140.
Voogd TE, Vansterkenburg ELM, Wilting J and Janssen LHM (1993) Recent research on the biological activity of suramin. *Pharmacol Rev* **45**:177–203.
Vulchanova L, Arvidsson U, Riedl M, Wang J, Buell G, Surprenant A, North RA and Elde R (1996) Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry. *Proc Natl Acad Sci USA* **93**:8063–8067.
Vulchanova L, Riedl MS, Shuster SJ, Buell G, Surprenant A, North RA, and Elde R

(1997) Immunohistochemical study of the $P2X_2$ and $P2X_3$ receptor subunits in rat and monkey sensory neurons and their central terminals. *Neuropharmacology* **36**:1229–1242.

Waldo GL, Boyer JL, Morris AJ and Harden TK (1991a) Purification of an AlF_4- and G-protein $\beta\gamma$-subunit-regulated phospholipase C-activating protein. *J Biol Chem* **261**:14217–14225.

Waldo GL, Morris AJ, Klapper DG and Harden TK (1991b) Receptor and G-protein regulated 150-kDa avian phospholipase C: Inhibition of enzyme activity by isoenzyme specific antisera and nonidentity with mammalian phospholipase C isoenzymes established by immunoreactivity and peptide sequence. *Mol Pharmacol* **40**:480–489.

Walker BA, Hagenlocker BE, Douglas VK, Tarapchak SJ and Ward PA (1991) Nucleotide responses of human neutrophils. *Lab Invest* **64**:105–112.

Walker BA, Jacobson MA, Knight DA, Salvatore CA, Weir T, Zhou D and Bai TR (1997) Adenosine A_3 receptor expression and function in eosinophils. *Am J Resp Cell Mol Biol* **16**:531–537.

Walker BAM, Rocchini C, Boone RH, IP S and Jacobson MA (1996) Adenosine A_{2a} receptor activation delays apoptosis in human neutrophils. *J Immunol* **158**:2926–2931.

Wang C-Z, Namba N, Gonoi T, Inagaki N and Seino S (1996) Cloning and pharmacological characterization of a fourth P2X receptor subtype widely expressed in brain and peripheral tissues including various endocrine tissues. *Biochem Biophys Res Commun* **220**:196–202.

Wang J, Drake L, Sajjadi F, Firestein GS, Mullane KM and Bullough DA (1997) Dual activation of adenosine A1 and A_3 receptors mediates preconditioning of isolated cardiac myocytes. *Eur J Pharmacol* **320**:241–248.

Warland JJI and Burnstock G (1987) Effects of reserpine and 6-hydroxydopamine on the adrenergic and purinergic components of sympathetic nerve responses of the rabbit saphenous artery. *Br J Pharmacol* **92**:871–880.

Wax M, Sanghavi DM, Lee CH and Kapadia M (1993) Purinergic receptors in ocular ciliary epithelial cells. *Exp Eye Res* **57**:89–95.

Webb RL, Sills MA, Chovan JP, Peppard JV and Francis JE (1993a) Development of tolerance to the antihypertensive effects of highly selective adenosine A_{2a} agonists upon chronic administration. *J Pharmacol Exp Ther* **267**:287–295.

Webb TE, Boluyt MO and Barnard EA (1996d) Molecular biology of P_{2Y} purinoceptors: Expression in heart. *J Auton Pharmacol* **16**:303–307.

Webb TE, Feolde E, Vigne P, Neary JT, Runberg A, Frelin C and Barnard EA (1996c) The P2Y purinoceptor in rat brain microvascular endothelial cells couple to inhibition of adenylate cyclase. *Br J Pharmacol* **119**:1385–1392.

Webb TE, Henderson D, King BF, Wang S, Simon J, Bateson AN, Burnstock G and Barnard EA (1996a) A novel G protein-coupled P_2 purinoceptor ($P2Y_3$) activated preferentially by nucleoside diphosphates. *Mol Pharmacol* **50**:258–265.

Webb TE, Kaplan MG and Barnard EA (1996b) Identification of 6H1 as a P2Y purinoceptor: $P2Y_5$. *Biochem Biophys Res Commun* **219**:105–110.

Webb TE, King BF, Burnstock G and Barnard EA (1995) Cloning and expression of a novel P_2 purinoceptor: $P2Y_3$. *FEBS Abstracts* **21**:P27.

Webb TE, Simon J, Bateson AN and Barnard EA (1994) Transient expression of the recombinant chick brain P_{2y1} purinoceptor and localization of the corresponding mRNA. *Cell Mol Biol (Noisy-Le-Grand)* **40**:437–442.

Webb TE, Simon J, Krishek BJ, Bateson AN, Smart TG, King BF, Burnstock G and Barnard EA (1993b) Cloning and functional expression of a brain G-protein-coupled ATP receptor. *FEBS Lett* **324**:219–225.

Weinberg JM, Davis JA, Shayman JA and Knight PR (1989) Alterations of cytosolic calcium in $LL\text{-}PK_1$ cells induced by vasopressin and exogenous purines. *Am J Physiol* **256**:C967–C976.

Weisman GA, De BK and Pritchard RS (1989) Ionic dependence of the extracellular ATP-induced permeabilization of transformed mouse fibroblasts: Role of plasma membrane activities that regulate cell volume. *J Cell Physiol* **138**:375–383.

Werner P, Stewart E, Buell G and North RA (1996) Domains of P2X receptors involved in desensitization: *Proc Natl Acad Sci USA* **93**:15485–15490.

Westfall DP, Hogaboom GK, Colby J, O'Donnell JP and Fedan JS (1982) Direct evidence against a role for ATP as the non-adrenergic, non-cholinergic inhibitory neurotransmitter in the guinea-pig taenia coli. *Proc Natl Acad Sci USA* **79**:7041–7045.

White TE, Dickenson JM, Alexander SPH and Hill SJ (1992) Adenosine A_1-receptor stimulation of inositol phospholipid hydrolysis and calcium mobilisation in DDT_1 MF-2 cells. *Br J Pharmacol* **106**:215–221.

Wickman K and Clapham DE (1995) Ion channel regulation by G proteins. *Physiol Rev* **75**:865–885.

Wiklund NP and Gustafsson LE (1988) Indications for P_2-purinoceptor subtypes in guinea-pig smooth muscle. *Eur J Pharmacol* **148**:361–370.

Wildman SS, King BF and Burnstock G (1997) Potentiation of ATP-responses at a recombinant $P2X_2$ receptor by neurotransmitters and related substances. *Br J Pharmacol* **120**:221–224.

Wiley JS, Chen JR, Snook MS, Gargett CE and Jamieson GP (1996) Transduction mechanisms of P2Z purinoceptors, in *P2 Purinoceptors: Localization, Function and Transduction Mechanisms* (Chadwick DJ and Goode JA eds) pp 149–165, John Wiley & Sons, Chichester.

Wiley JS, Chen JR, Snook MB and Jamieson GP (1994) The P_{2Z}-purinoceptor of human lymphocytes: Actions of nucleotide agonists and irreversible inhibition by oxidized ATP. *Br J Pharmacol* **112**:946–950.

Wiley JS, Chen R and Jamieson GP (1993) The ATP^{4-} receptor-operated channel (P_{2Z} class) of human lymphocytes allows Ba^{2+} and ethidium$^+$ uptake: Inhibition of fluxes by suramin. *Arch Biochem Biophys* **305**:54–60.

Wilkinson GF, Purkiss JR and Boarder MR (1994) Differential heterologous and homologous desensitization of two receptors for ATP (P2y receptors and nucleotide receptors) coexisting on endothelial cells. *Mol Pharmacol* **45**:731–736.

Williams M (1989) Adenosine antagonists as therapeutic agents. *Med Res Rev* **9**:219–243.

Windscheif U (1996) Purinoceptors: From history to recent progress: A review. *J Pharm Pharmacol* **48**:993–1011.

Windscheif U, Pfaff O, Ziganshin AU, Hoyle CHV, Bäumert HG, Mutschler E, Burnstock G and Lambrecht G (1995a) Inhibitory action of PPADS on relaxant responses to adenine nucleotides or electrical field stimulation in guinea-pig taenia coli and rat duodenum. *Br J Pharmacol* **115**:1509–1517.

Windscheif U, Radziwon P, Breddin HK, Bäumert H, Lambrecht G and Mutschler E (1995b) Two different inhibitory effects of pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid on adenosine diphosphate-induced human platelet aggregation. *Arzneimittel-Forsch* **45**:994–997.

Windscheif U, Ralevic V, Bäumert HG, Mutschler E, Lambrecht G and Burnstock G (1994) Vasoconstrictor and vasodilator responses to various agonists in the rat perfused mesenteric arterial bed: Selective inhibition by PPADS of contractions mediated via P2X-purinoceptors. *Br J Pharmacol* **113**:1015–1021.

Wittenburg H, Bültmann R, Pause B, Ganter C, Kurz G and Starke K (1996) P_2-purinoceptor antagonists II. Blockade of P_2-purinoceptor subtypes and ecto-nucleotidases by compounds related to Evans blue and trypan blue. *Naunyn-Schmiedeberg's Arch Pharmacol* **354**:491–497.

Wolkoff LI, Perrone RD, Grubman SA, Lee DW, Soltoff SP, Rogers LC, Beinhorn M, Fang SL, Cheng SH and Jefferson DM (1995) Purinoceptor P_{2U} identification and function in human intrahepatic biliary epithelial cell lines. *Cell Calcium* **17**:375–383.

Wright JM and Li C (1995) Zn^{2+} potentiates steady-state ATP activated currents in rat nodose ganglion neurons by increasing the burst duration of a 35 pS channel. *Neurosci Lett* **193**:177–180.

Wu LG and Saggau P (1994) Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. *Neuron* **12**:1139–1148.

Yagil Y (1994) The effects of adenosine on water and sodium excretion. *J Pharmacol Exp Ther* **268**:826–835.

Yagil C, Katni G and Yagil Y (1994) The effects of adenosine on transepithelial resistance and sodium uptake in the inner medullary collecting duct. *Pflüeg Arch Eur J Physiol* **427**:225–232.

Yakel JL, Warren RA, Reppert SM and North RA (1993) Functional expression of adenosine A_{2b} receptor in *Xenopus* oocytes. *Mol Pharmacol* **43**:277–280.

Yamaguchi M, Hirayoshi K, Okuma M and Nagata K (1994) Enhancement of differentiation induction of mouse myelomonocytic leukemic cells by extracellular ATP. *J Cell Physiol* **159**:441–449.

Yang C-M, Tsai Y-J, Pan S-L, Tsai C-T, Wu W-B, Chiu C-T, Luo S-F and Ou JT (1997) Purinoceptor-stimulated phosphoinositide hydrolysis in Madin-Darby canine kidney (MDCK) cells. *Naunyn-Schmiedeberg's Arch Pharmacol* **356**:1–7.

Yang S, Buxton ILO, Probert CB, Talbot JN and Bradley ME (1996) Evidence for a discrete UTP receptor in cardiac endothelial cells. *Br J Pharmacol* **117**:1572–1578.

Yang S, Cheek DJ, Westfall DP and Buxton IL (1994) Purinergic axis in cardiac blood vessels: Agonist-mediated release of ATP from cardiac endothelial cells. *Circ Res* **74**:401–407.

Yao Y, Yoshitatsu S, Abbracchio MP, Jiang J-L, Kim Y-C and Jacobson KA (1997) Adenosine A_3 receptor agonists protect HL-60 and U-937 cells from apoptosis induced by A3 antagonists. *Biochem Biophys Res Commun* **232**:317–322.

Yao Z and Gross GJ (1993) Glibenclamide antagonizes adenosine A_1 receptor-mediated cardioprotection in stunned canine myocardium. *Circulation* **88**:235–244.

Yokomizo T, Izumi T, Chang K, Takuwa Y and Shimizu T (1997) A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis. *Nature (Lond.)* **387**:620–624.

Yu HX and Turner JT (1991) Functional studies in the human submandibular duct cell line, HSG-PA, suggest a second salivary gland receptor subtype for nucleotides. *J Pharmacol Exp Ther* **259**:1344–1350.

Yu S-M, Chen S-F, Lau Y-T, Yang C-M and Chen J-C (1996) Mechanism of extracellular ATP-induced proliferation of vascular smooth muscle cells. *Mol Pharmacol* **50**:1000–1009.

Zahler S, Becker BF, Raschke P and Gerlach E (1994) Stimulation of endothelial adenosine A_1 receptors enhances adhesion of neutrophils in the intact guinea pig coronary system. *Cardiovasc Res* **28**:1366–1372.

Zegarra-Moran O, Romeo G and Galietta LJ (1995) Regulation of transepithelial ion transport by two different purinoceptors in the apical membrane of canine kidney (MDCK) cells. *Br J Pharmacol* **114**:1052–1056.

Zetterström T and Fillenz M (1990) Adenosine agonists can both inhibit and enhance in vivo striatal dopamine release. *Eur J Pharmacol* **180**:137–143.

Zhang G, Miyahara H and Suzuki H (1989) Inhibitory actions of adenosine differ between ear and mesenteric arteries in the rabbit. *Pflüeg Arch Eur J Physiol* **415**:56–62.

Zhang Y, Palmblad J and Fredholm BB (1996) Biphasic effect of ATP on neutrophil functions mediated by P_{2U} and adenosine A_{2A} receptors. *Biochem Pharmacol* **51**:957–965.

Zhang Y and Wells JN (1990) The effects of chronic caffeine administration on peripheral adenosine receptors. *J Pharmacol Exp Ther* **254**:757–763.

Zhang YX, Yamashita H, Oshita T, Sawamoto N and Nakamura S (1995) ATP increases extracellular dopamine level through stimulation of P_{2Y} purinoceptors in the rat striatum. *Brain Res* **691**:205–212.

Zhou X and Galligan JJ (1996) P_{2X} purinoceptors in cultured myenteric neurons of guinea-pig small intestine. *J Physiol (Lond)* **496**:719–729.

Zhou QY, Li C, Olah ME, Johnson RA, Stiles GL and Civelli O (1992) Molecular cloning and characterization of an adenosine receptor: The A_3 adenosine receptor. *Proc Natl Acad Sci USA* **89**:7432–7436.

Zhu Y and Ikeda SR (1993) Adenosine modulates voltage-gated Ca^{2+} channels in adult rat sympathetic neurons. *J Neurophysiol* **70**:610–620.

Ziganshin AU, Hoyle CHV, Bo X, Lambrecht G, Mutschler E, Bäumert HG and Burnstock G (1993) PPADS selectively antagonizes P_{2X}-purinoceptor-mediated responses in the rabbit urinary bladder. *Br J Pharmacol* **110**:1491–1495.

Ziganshin AU, Hoyle CHV and Burnstock G (1994a) Ecto-enzymes and metabolism of extracellular ATP. *Drug Dev Res* **32**:134–146.

Ziganshin AU, Hoyle CHV, Lambrecht G, Mutschler E, Bäumert HG and Burnstock

G (1994b) Selective antagonism by PPADS at P_{2X}-purinoceptors in rabbit isolated blood vessels. *Br J Pharmacol* **111**:923–929.

Ziganshin AU, Ziganshin LE, King BF and Burnstock G (1995) Characteristics of ecto-ATPase of *Xenopus* oocytes and the inhibitory actions of suramin on ATP breakdown. *Pflüeg Arch Eur J Physiol* **429**:412–418.

Zimmermann H (1996) Biochemistry, localization and functional roles of ecto-nucleotidases in the nervous system. *Prog Neurobiol* **49**:589–618.

Ziyal R (1997) *Pharmacological evaluation of the suramin analog NF023 as a P_2-purinoceptor antagonist at vascular and non-vascular preparations in vitro and as an inhibitor of the ecto-nucleotidases* [PhD thesis]. Aachen, Shaker.

Ziyal R, Ziganshin AU, Nickel P, Ardanuy U, Mutschler E, Lambrecht G and Burnstock G (1997) Vasoconstrictor responses via P2X-receptors are selectively antagonized by NF023 in rabbit isolated aorta and saphenous artery. *Br J Pharmacol* **120**:954–960.

Zocchi C, Ongini E, Ferrara S, Baraldi PG and Dionisotti S (1996) Binding of the radioligand [^{3}H]-SCH 58261, a new non-xanthine A_{2A} adenosine receptor antagonist, to rat striatal membranes. *Br J Pharmacol* **117**:1381–1386.

Zoetewij JP, Van De Water B, De Bont HJ and Nagelkerke JF (1996) The role of a purinergic P_{2Z} receptor in calcium-dependent cell killing of isolated rat hepatocytes by extracellular adenosine triphosphate. *Hepatology* **23**:858–865.